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TANNIC ACID AND IRON ALUM WITH SAFRANIN AND ORANGE G IN STUDIES OF THE SHOOT APEX

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ABSTRACT.—**A schedule is given for staining the cell walls of young plant tissues in tannic acid and iron alum after the protoplasts have been stained in safranin and orange G. Sections are placed for one minute in 2% aqueous $ZnCl_2$, and are then stained in a 1/25,000 aqueous solution of safranin O. From this they are placed for five minutes in a bath consisting of orange G (2 g.), tannic acid (5 g.), water (up to 100 cc.) and HCl (4 drops). This is followed by five minutes in 5% aqueous tannic acid and two minutes in a 1% solution of iron alum. A brief rinse in tap water is given between each stage; the slides are raised and lowered about a dozen times at each change to ensure that the new solution reaches the material quickly. The method was originated for shoot apices but it also works excellently on more mature tissues and on adult material. It has the advantage of allowing extremely easy detection of protophloem in the strands even at the very onset of vascular differentiation.**

Sections of the shoot apex rarely allow critical observations to be made on the more detailed aspects of their architecture when stained by the usual methods. This region of small cells with denser, less vacuolated protoplasts and thin walls, needs a special technic very different from that applicable to the more differentiated regions. In spite of the fact that the tannic-acid-iron-chloride method of Rösler (1928), Zimmermann (1928), Korody (1937), Härtel (1938) etc. has the great advantage of showing up the cell walls even in the extreme apex, the results obtained always appear a little monotonous, so that there is always felt a need for some counterstain which will pick out the protoplasts as well as facilitate observations on the stages in the differentiation of the vascular strands. The use of safranin in the manner suggested by Foster (1934) results in an inordinately long schedule of over 48 hours; it is difficult to stain the protoplasts once the sections have been carried thru the normal tannic-acid-iron

schedule; on the other hand, it is also difficult to restrict most stains to the protoplasts so as to leave the cell walls to be stained by the iron tannate.

During investigations on the organization of the shoot in the grasses, the difficulties involved led to the use of the following rather unorthodox technic which may be of use to others interested in plant anatomy. The success of the method rests on the preliminary staining of the cell contents by the use of a very dilute aqueous solution of safranin O (1 or 2 parts in 25,000 of water) followed by an acid solution of orange G. This results in the almost exclusive staining of the cell contents and lignified walls, leaving all unlignified walls almost uncolored. Once the protoplasts have been stained in this way, they appear to lose their affinity for the tannic acid (possibly owing to the saturation of the protein side chains); thus when the sections are placed in the iron solution the protoplasts do not become darkened but remain orange or yellow and show up in good contrast with the blue-black of the cell walls. The method may be used in combination with most fixatives, but gives the best results when used after formalin-fatty-acid or formalin-fatty-acid-alcohol fixatives of the type of Chamberlain (1935), Juel [see Zimmermann, W. A. (1928), Korody, E. (1937) or Härtel, K. (1938)] or the common modifications of these [see Ball, E. (1941)]. After the Flemming or chrom-acetic type of fixatives, or on material which has been fixed in a formalin-fatty-acid mixture and then post chromatized, the protoplasts only stain very lightly, altho the cell walls are still stained fairly well. Of course none of the above fixatives give any representation of the vacuolation of the extreme apical cells; unfortunately, vacuome fixatives do not lend themselves at all well to a critical study of the architecture of the shoot apex. Vacuolation must be studied in material fixed as well as stained with that end in view.

Altho the method to be described was originally worked out for apical tissues using sections cut at 4–6 μ, the clarity with which it shows the early as well as late stages in the development of the vascular strands, and the sharp tissue differentiation which it gives thruout the material, make it very useful for more mature parts. The writer has used it, for instance, with considerable success on 10–15μ sections in a study of the adult anatomy of abnormal flowers in *Zea*.

The schedule is as follows:

1. Take the slides down to water (tap water is quite satisfactory).
2. Treat 1 min. in 2% aqueous $ZnCl_2$.
3. Rinse about 5 sec. in tap water.

4. Stain 5 min. in 1:25,000 aqueous safranin[1].
5. Rinse about 5 sec. in tap water.
6. Stain 1 min. in the following solution: orange G[1], 2 g.; tannic acid[1], 5 g.; HCl (conc.), 4 drops; thymol, a few crystals; water, to make 100 cc.
7. Rinse about 5 sec. in tap water.
8. Treat 5 min. in the following: tannic acid, 5 g.; thymol, a few crystals; water, to make 100 cc.
9. Rinse quickly (1–3 sec.) in tap water.
10. Treat 2 min. in 1% aqueous iron alum.
11. Rinse 15 sec. in tap water.
12. Place for 5 sec. in 45% alcohol and for 5–10 sec. in 90% alcohol. Wipe excess off slide.
13. Treat 10 sec. in absolute alcohol. Wipe excess off slide.
14. Immerse for 20–30 sec. in: clove oil, 1 vol.; xylene, 3 vol. Wipe slide.
15. Place for 20–30 sec. in xylene.
16. Mount in balsam.

If it is desired to simplify the schedule slightly, the following stage may be inserted in place of stages 6, 7, and 8:

6a. Stain 5 min. in the following: orange G, 0.5 g.; tannic acid, 10 g.; HCl (conc.), 4 drops; thymol, a few crystals; water, to make 100 cc. Then pass to stage 9.

Altho this modification works well with more mature tissues, with apical meristems it does not give quite such good results as the longer method; the cell walls are not so well stained and less control can be exercized over the staining of the protoplasts.

The following notes may help. The ribbon may be floated out in the normal way, tho the writer prefers to use a dilute solution of safranin in place of the usual distilled water (1 cc. of a 2% solution of safranin to ½–1 liter of water is about the right concentration). The exact strength is not very important; once a solution has been made and its depth of color noted, any needed in the future can easily be produced by approximating the color. Sections floated out on this solution quickly take up the dye and, in spite of their still being in wax, show considerable detail when examined under the microscope, with the result that those which do not include the desired portion of the material may be discarded at once. After expansion, the sections are dried in the usual manner. When dry, the slides are

[1]The writer uses "Revector" safranin O and orange G; no doubt stains from any reputable firm may be used for the method. The tannic acid was the normal commercial type.

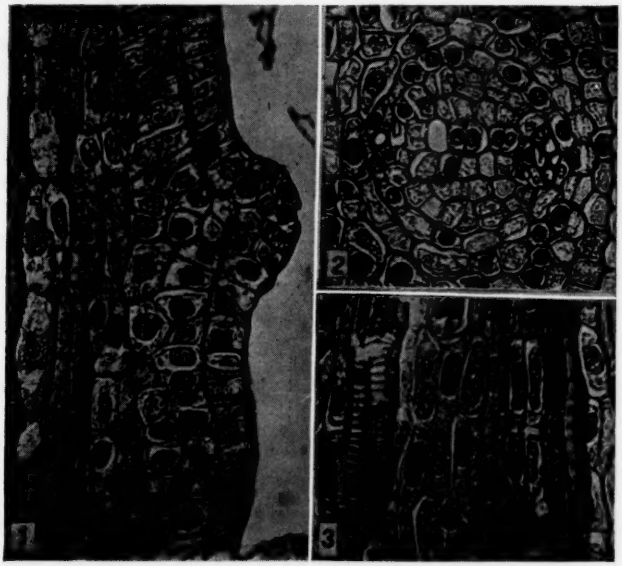
warmed, taken into xylene and down the alcohols as in normal practice. Safranin will be observed streaming off the slides and out of the sections as they are taken down the alcohols; they are left in the 45% alcohol until they are colorless or at least only just pink. It is so difficult to stop the removal of safranin at the required stage that it is necessary to remove it all and then restain in the dilute solution at stage 4.

The writer finds three grades of alcohol (absolute, 90%, and 45%) quite sufficient when taking the slides to and from water. When placing slides in any solution, it is a good practice to raise and lower them about a dozen times as this ensures that the new solution quickly replaces the old. This method affords a considerable saving in time and the above schedule is drawn up on the assumption that it is applied at each stage. (The effect can easily be illustrated by taking a plain slide from a dye solution and placing it in a tube of clean water—it is surprising the length of time which elapses before all the stain leaves the slide by diffusion alone: raising and lowering the slide a few times accomplishes as much as the first minute's standing). Using this method, the tap water rinses may be cut down to about five seconds each.

The safranin for stage 4 may conveniently be prepared by adding about 2 cc. of a stock 2% aqueous solution to a liter of water. The $ZnCl_2$ is made by dissolving a piece of stick of about 2 g. in 100 cc. of water and then filtering off the white precipitate which appears. The solution need only be approximately 2%. Without $ZnCl_2$, the walls at the apical region are difficult to stain. When going from the orange G solution to the tannic acid, the quick rinse in tap water will usually not remove the whole of the excess dye, but the small amount caried over will do no harm. Both the orange G and the tannic acid of stage 8 should have a few crystals of thymol (or sodium salicylate) added to prevent the growth of fungi in them. The tannic acid solution is originally a pale straw color but gradually assumes a brownish tint (oxidation?); however, its staining capacity is not affected. The iron alum need only be approximately 1%; noting the amount of crystals used when the solution is made up for the first time will enable any to be made up later without weighing. When a slide is first placed in the iron alum there may be a tendency for the formation of a *small* cloud of black iron tannate in the liquid, but moving the slide about will prevent this from settling on the material and after the first few seconds it seems to dissolve in the alum solution.

There does not appear to be any advantage in leaving the sections longer in the alcohol stages when going up the series after staining;

in fact definite harm is more likely to be done since a prolonged stay in the lower alcohols may tend to remove the stains from the protoplasts. The clove-oil-xylene mixture at stage 14 will act as a safeguard against the ill effects of the small amount of water which will almost certainly be carried up. If any cloudiness is observed at this point, a few drops of absolute alcohol may be added. Two changes



Sections of *Agropyron repens* Beauv., stained by the procedure described. ×500.

FIG. 1. Longitudinal section of apical region, showing the origin of a new leaf primordium on the side of the apex.

FIG. 2. Transverse sections of a young median leaf trace bundle with one protoxylem and five protophloem elements.

FIG. 3. Longitudinal section of a young median leaf trace bundle to show the ease with which protophloem elements may be distinguished from the surrounding cells. (Kodak 0 120 plate, using a filter made by dyeing a fixed blank plate in 2% aqueous orange G.)

of xylene at the finish are an advantage, altho one alone, as in the schedule, is quite sufficient.

In the resulting sections, the cell walls should be stained blue-black; there should be no difficulty in staining even those at the extreme apex. The nuclei should appear shades of yellow to orange, varying according to their metabolism. The staining of the cyto-

plasm will appear different in cells at different parts. Usually it will be pale yellow but in actively dividing cells, in the procambium or at the tips of young leaves, for instance, it is generally a darker yellow or orange. Procambial cells about to differentiate to protophloem often show dense brilliant orange contents, whilst the protophloem tubes themselves are exceedingly well defined and appear dark blue or black contrasting well with the rest of the cells of the strand; the numerous pits on their side walls show up very clearly. The color of the cytoplasm in older tissues varies from gray in the pith parenchyma cells to orange in the developing mesophyll of the leaf. In the former tissue the dark walls make the pits very conspicuous. Starch grains, in material fixed to preserve them, stain black and, being easily picked out, may be observed in many insteresting places. In the rhizome tips of *Agropyron repens* Beauv., for example, they are clearly seen in the central cells of the apical cone itself, some distance above the level of the insertion of the first primordium.

In the strands, the phloem elements have black walls with orange contents. The coloring of the xylem elements depends on their degree of lignification: young unlignified walls appear blue-gray, partially lignified ones various shades of violet, mauve or pink, whilst fully lignified ones are a cherry red. Mature sclerenchyma elements are also red.

Fig. 1 is a longitudinal section of a shoot of *Agropyron repens* Beauv., showing the origin of a new primordium on the side of the apex. Fig. 2 and 3 are a transverse and longitudinal section, respectively, of a median trace bundle of the same species to show the ease with which the protophloem may be distinguished from the procambial and surrounding cells, and the type of staining given by tissues a little way back from the apex; (unfortunately, in the photograph shown in Fig. 3 the pits on the "back" walls of the protophloem elements do not show as they are out of focus).

The staining is permanent; some preparations two or three years old still appear in their original condition. The writer has always dried slides stained in this way in an oven at about 40°C. but has not seen any signs of fading. Sections stained by this method are very pleasant to work with because their coloring enables them to be examined for long periods without fatigue as well as rendering them excellent for photographing.

REFERENCES

Ball, E. 1941. Microtechnique for the shoot apex. Amer. J. Bot., 28, 233–43.

Chamberlain, C. J. 1935. Methods in plant histology. Univ. of Chicago Press, Chicago.

FOSTER, A. S. 1934. The use of tannic acid and iron chloride for staining cell walls in meristematic tissue. Stain Techn., 9, 91–2.

HÄRTEL, K. 1938. Studien an Vegetationspunkten einheimischer Lycopodien. Beitr. Biol. Pfl., 25, 125–68.

KORODY, E. 1937. Studien am Spross-Vegetationspunkt von Abies concolor, Picea excelsa und Pinus montana. Beitr. Biol. Pfl., 25, 23–59.

RÖSLER, P. 1928. Histologische Studien am Vegetationspunkt von Triticum vulgare. Planta, 5, 28–69.

ZIMMERMANN, W. A. 1928. Histologische Studien am Vegetationspunkt von Hypericum uralum. Jahr. wiss. Bot., 68, 289–344.

CURDLED MILK FOR SUPPORTING TISSUES IN CELLOIDIN EMBEDDING

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ABSTRACT.—**To make a material for supporting tissues during celloidin embedding, milk curd is hardened in ordinary strong alcohol, cut into suitable shapes, dehydrated, extracted with ether, and preserved indefinitely in 1:1 alcohol-ether. The material, which consists of the precipitated proteins of milk, is embedded and sectioned with the tissue. It presents no detectable opposition to the passage of the microtome-knife.**

There are several reasons why it is often desirable to support tissues while the celloidin in which they are being embedded is becoming transformed into a gel. If the tissue is simply allowed to lie on the bottom of the embedding-chamber, a part of it will necessarily coincide with the edge of the finished block. This is to be avoided, because the outer edge of the block generally becomes harder than the rest, and it is convenient to slice this harder part off and thus leave a homogeneous piece of celloidin. Again, when $K_2Cr_2O_7$ and certain other fixatives are used, the tissue sometimes becomes crumbly and also tends to adhere to the bottom of the embedding-chamber, so that when the block is removed, part of the tissue tends to be torn off. Apart from the desirability of holding the tissue away from the bottom of the embedding-chamber, it is often necessary to orientate the tissue in a special position, and this especially applies when two or more pieces are being embedded in the same block (e.g., for the preparation of longitudinal and transverse sections at the same time). Workers with celloidin know how this substance, when passing from sol to gel on evaporation, tends to move objects lying in it, and it is clearly advantageous in several ways to have some kind of support.

Apáthy long ago (1888) put plates of gelatin (of the kind used by lithographers) underneath tissues during celloidin embedding, and subsequently sectioned the gelatin with the object. His purpose, however, was rather to mark the orientation of the object than to support it. Halle (1895) used a mixture of egg-white and egg-yolk prepared and hardened by a special method devised for a different purpose by Calberla (1876). Halle preserved Calberla's egg-mass in absolute alcohol, and brought it into weak celloidin solution when

required. The egg-mass was embedded with the object. Eycleshymer (1892) devised several methods. Sometimes he supported tissues on pins pushed thru the sides of paper embedding-boxes. Sometimes he thrust pins longitudinally thru a bottle-cork, transfixed the objects to be embedded on the ends of the pins, and wrapped paper round the cork in such a way as to make it the bottom of a cylindrical embedding-chamber. At other times he used a metal embedding chamber with small holes in the sides thru which silk threads were passed to support the object. The pins or thread were pulled out when the celloidin had hardened. All these methods present disadvantages. Embedding chambers of glass or porcelain are more convenient than those of paper, or paper and cork; and silk threads do not support tissues as firmly as solid blocks of material. The writer's attempts to make a suitable preparation of gelatin for the purpose have not been successful, and Calberla's egg-mass is unnecessarily troublesome to prepare.

The writer's purpose has been to improve on these methods. He has sought to find a suitable material to embed with the tissue to hold it in position while the celloidin is hardening. Such a material should be easily prepared, capable of being stored indefinitely and of being cut into suitable shapes, easily penetrated by celloidin, and so soft as to present no detectable resistance to the microtome-knife. Such a substance is ordinary milk curd, hardened in alcohol, extracted with ether and stored in 1:1 alcohol-ether.

Liquid extract of rennin is added to fresh milk in the proportion of about 1 cc. to 100 cc. The milk is maintained at 35°–45° C. until a firm curd has set. Lumps of the curd are then transferred to strong alcohol (undiluted industrial alcohol, about 94%) by means of a tablespoon, 100 cc. of alcohol being allowed to each tablespoonful. The spoon must be manipulated gently so as not to crack the lumps of curd which it transfers, and the vessel containing the alcohol should be left unmoved after the curd has been transferred to it. It is best to harden for about a week, using three lots of strong alcohol. When changing the alcohol, care must be taken not to crack the curd. It is best to remove the used alcohol with a siphon, and thus avoid any movement of the vessel. At the end of a week the hardened curd is cautiously cut with a safety razor blade into pieces of any suitable shape and size. For general purposes brick-shaped pieces about 2 cm. long will be found convenient. The pieces are next dehydrated in three successive lots of absolute alcohol and then passed thru two lots of ether to dissolve all fats and leave only the precipitated milk-proteins behind. The periods in absolute alcohol and ether will

naturally depend on the size of the pieces. There is no harm (but no benefit) in leaving them for long periods, such as a week, in these fluids. The pieces are then transferred from ether to 1:1 alcohol-ether and stored in this indefinitely. The prepared curd is very friable, but one soon learns to handle it with sufficient care to prevent breaking or crumbling.

When the specimen which is being embedded is transferred from alcohol-ether to celloidin solution, a brick of the curdled milk is transferred at the same time to the same vessel. When the hardening of the celloidin is about to begin—whether by evaporation or treatment with chloroform—the object is arranged so as to rest in the required position on the brick. If the object is large, one may use several bricks; if it is small, a minute hole or groove may be hollowed out in the upper surface of the brick to receive it by scraping gently with a pin or the point of a scalpel. When the celloidin has been hardened, the block is trimmed. This may or may not involve the cutting away of part of the curd. The remainder of the curd is cut with the object. It presents no detectable opposition to the smooth passage of the microtome-knife.

The curdled milk is white and clearly visible thru the celloidin. If it is wished to color it, to help in the orientation of the object, the bricks are stained overnight in paracarmine and then washed in several changes of acid alcohol before dehydrating in absolute alcohol and soaking in ether. The red bricks, like the white ones, are stored in 1:1 alcohol-ether.

REFERENCES

Apáthy, S. 1888. Nachträge zur Celloidintechnik. Zts. wiss. Mikr., **5**, 45–9.

Calberla, E. 1876. Eine Einbettungsmasse. Morph. Jahrb., **2**, 445–8.

Eycleshymer, A. C. 1892. Notes on celloidin technique. Amer. Nat., **26**, 354–7.

Halle (initials not given). 1895. Ueber die Herstellung von plastichen Structurbildern der Haut nach der Plattenmodellirmethode. *Abstract in* Zts. wiss. Mikr., **12**, 364–6.

ROOT-TIP SMEARS FOLLOWING FIXATION WITH BOILING WATER

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ABSTRACT.—**A convenient, quick method of preparing fresh root-tips for the detailed study of the critical stages of mitosis is presented. The cells of fresh-cut root-tips are killed and fixed instantaneously by immersion in boiling water. To effect maceration, the root-tips are transferred to a mixture of 95% ethyl alcohol and concentrated HCl. The root-tips are then smeared in a drop of aceto-carmine with glass instruments. Application of mineral oil makes the preparations permanent or semi-permanent. Characteristically, anaphasic chromosomes in the fresh materials thus prepared appear as structures composed of a swollen transparent matrix in which are embedded spiral interlocking chromonemata. Suggestions are offered as to the advantages and possibilities of the technic.**

Since the days of Sakamura's work on the pollen-mother cells of *Tradescantia virginica* and his emphasis on the desirability of observation on fresh material, little has been done to project the essentials of his method to other species or to somatic mitosis. It is thought by the present author that additional information and insight as to the character of the repeatedly-observed chromonematic spirals may be gained by the study of cells prepared by the following method:

Method. 1. To kill and to fix tissues, cut off the root-tip[1] and immerse immediately in boiling water in a small beaker. 2. After one minute, remove the root-tip and allow it to cool on a glass slide. 3. Cover the tip with a drop or two of a solution consisting of equal parts of 95% ethyl alcohol and concentrated HCl. This solution dissolves the middle lamella. 4. Remove the softened root-tip to another glass slide, add a drop of aceto-carmine, and with the smooth convex surface of a small watch glass, macerate by gentle pressure and a rocking motion. The progress of the operation may be delicately controlled visually and tactually. 5. Cover with a cover glass. 6. If, when the preparation is held to the light, aggregations of cells are obvious, disperse them by applying local pressure upon the cover glass. 7. Prevent the preparation from drying out by ringing the cover glass with mineral oil.[2] The mineral oil works to

[1]The author worked with root-tips of *Vicia faba* and *Allium cepa*.

[2]The mineral oil used is sold under the trade name of "Nujol".

best advantage when it displaces the aceto-carmine solution and surrounds individual cells.

Results. Chromosomes treated by this method swell considerably. Such increase in volume is correlated with a diminution of chromaticity, so that in metaphase and anaphase they appear as plump refractile matrices (not always distinct) in which are embedded two interlocking chromatic spirals. Matrices are not evident in prophase. These spirals at anaphase and telophase are widely separated, always regularly and precisely arranged. In portions of the longer chromosomes, as many as five or six turns have been observed. In metaphasic chromatids the spiral configuration may or may not be evident, but it is certainly intrinsic; the spiral is generally a tight one, presenting such an appearance as that of two pieces of yarn held together at their ends and twisted about one another. Special attention should be given the prophasic threads, which appear double, but which the author suspects have a quadruple nature. One should also notice the occasional crushed nucleus which shows projecting thread-like components. A careful study of these threads may reveal additional information concerning the chromonemata of the resting nucleus.

The spiral chromonemata appear to increase in thickness from metaphase to telophase, and even after the nuclear membrane forms they are retained for a short time in the orderly orientation of the telophasic chromosomes. It is evident that the two interlocking spirals of telophasic chromosomes lose this condition during the resting stage, for they emerge in prophase as parallel threads. Also characteristic of resting nuclei in these preparations is the presence of one or more large clear vesicles. The cytoplasm is often granular or vacuolated. After a period of time, however, the cytoplasm clears, provided that the immersion oil has effectively prevented the preparation from drying.

The results of this method, including the existence and orderly double spiral character of the chromonemata, and the composition of the resting nucleus, deserve further study. It is entirely possible that critical observation and interpretation of such material may go far to supplement and correct previous views on the nature of the chromonemata and of the chromosome cycle in general.

A CONVENIENT METHOD FOR HANDLING CHICK EMBRYOS

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The following method is convenient and time saving in preparing large numbers of chick embryos for sectioning:

Cut several ordinary microscopic slides in half longitudinally with a glass cutter. Number each half slide with a diamond point pencil if the individual embryos are to be identified.

Remove the shell from the blunt end of the egg with a forceps, immerse in a finger-bowl half filled with 0.75% saline, and invert the shell, puring out the contents. The yolk will usually rotate the embryo to the top. With scissors cut a circular area around the embryo

FIG. 1.

Photograph of an 96-hour embryo cleared in cedarwood oil taken with an enlarger on Kodabromide F1 and developed in D72. The embryo was stained for sectioning and is too dark for a whole mount. The writer makes routine use of such photographs for interpretation of serial sections of experimental embryos.

about 2.5 cm. in diameter. After removing the vitelline membrane, grasp the membranes with a forceps and float the embryo onto a half slide held horizontally under the surface of the solution. Lifting the embryo partially out of the fluid, spread the membranes and arrange the embryo longitudinally in the center of the half slide. Holding this in a flat position, lift it out of the solution. (Surface tension forces will wrap the membranes around the glass). Invert, and place

a piece of filter paper 1×2 cm. on the membranes. Turn the half slide right side up and lay it horizontally in a petri dish filled with fixing fluid. (Fixation causes the membranes to adhere permanently to the filter paper holding the embryo against the glass.) After fixation transfer to a vessel of running water or to alcohol according to the fixing fluid used. Embryos thus attached may be conveniently transferred thru the various processes of staining, dehydration, clearing and even infiltration. The same containers used for processing mounted sections may also be used for embryos.

To examine or to photograph the embryos it is necessary to remove the filter paper and membranes from the back of the half slide. When dehydration is nearing completion, cut the membranes where they pass over the lower edge of the glass by pressing them against the edge, and remove the filter paper with forceps.

Altho the embryo may be detached at any stage, it is convenient to infiltrate with paraffin first. After clearing, transfer the embryo thru two changes of paraffin which may be kept in the same type of container as the other reagents. Without removing the half slide from the last paraffin bath, loosen the embryo with a warm needle and with a warm section lifter transfer it to the embedding paraffin.

The embryos are easily preserved by transferring the half slide to a dish of cold water after infiltration with paraffin. Preservation in paraffin permits the examination of the embryo with a binocular microscope and 50 such embryos can be stored in a 25-slide Pillsbury slide box. To embed for sectioning, replace the half slide in melted paraffin, and remove the embryo to the embedding paraffin after it has melted loose.

Embryos thus mounted can be quickly and easily photographed with a diffusion type photographic enlarger. Simply place the embryo which has been cleared in cedarwood oil on the negative holder and project it on bromide enlarging paper (Fig. 1). If the embryos have been stained in bulk for sectioning, a low contrast paper gives the best results. This method produces a negative image of the object but for record purposes it is as useful as a positive print. It is more economical of photographic emulsions and chemicals, which may be difficult to get, then the usual methods of photomicrography. Embryos up to 72 hours old and preserved in paraffin on a half slide may also be photographed successfully without removing the paraffin. Care should be taken not to overheat the embryos in the enlarger by using a large aperture and a short exposure.

DEMONSTRATING DOWNY MILDEW (BREMIA LACTUCAE) IN LETTUCE

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ABSTRACT.—**The leaf blade of young lettuce leaves, or an equivalent portion of the leaf blade of older leaves is placed dorsal side up in a Petri dish to which a moistened filter paper has been added. This leaf tissue is then inoculated with a suspension of conidia of the downy mildew organism. One drop of inoculum to each piece of tissue is sufficient. The material is then placed in a temperature chamber held at 12.5°C. After transfer to the temperature chamber the material can be removed at desired intervals for study.**

After fixation (3½–4 hours) in an acetic-acid-alcohol mixture (1:3), the fixing fluid is decanted and replaced with a solution of lactophenol-alcohol. It is then transferred to a clean, dry slide and flooded with 1% cotton blue (anilin blue W.S.) in 90% alcohol. The tissues of the host remain colorless, in contrast to the mycelium and germinating spore tube of the fungus which becomes dark blue as they come in contact with the stain.

As a preliminary step in an investigation designed to uncover the physiological basis for the difference in reaction between lettuce varieties susceptible to downy mildew (*Bremia lactucae* Reg.) and those which are immune, it was found necessary to devise a technic for studying the growth and development of the germinating conidia of the fungus and of the mycelium after its entrance into the host tissue. Since the cultural and staining technic used in this problem may be applicable to other parasitic fungi of the same general nature as *Bremia*, the procedures which have proved successful with this organism are described below.

A temperature chamber cooled to 12.5±0.5°C. is used for incubating the material. Experimental work has shown that this is about the optimum temperature for the most rapid growth and development of the fungus.[2] The host tissue employed usually consists of the entire leaf blade of very young leaves, or small portions of

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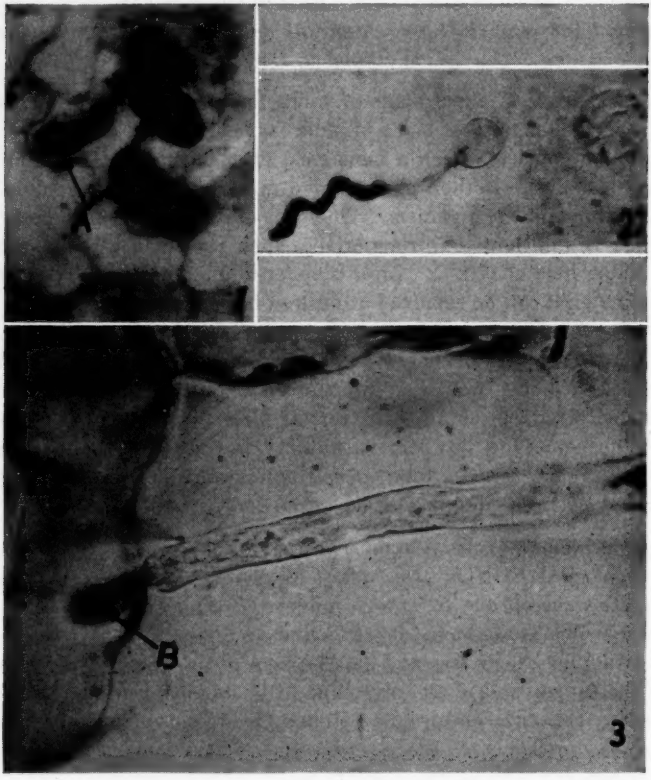
[2]Unpublished data.

the leaf blade of older leaves. The tissue is placed dorsal side up in a Petri dish to which a moistened filter paper has been added. It is then inoculated with a heavily charged suspension of conidia of the downy mildew organism. As a rule one drop of inoculum to each piece of tissue is sufficient.



Downy mildew (*Bremia lactucae*) in lettuce.

After transfer to the temperature chamber the material can be removed at suitable intervals for fixation, staining and subsequent study.

At the time of fixation, the leaf tissue is trimmed so that only the portion covered by the inoculum is placed in the fixing fluid. Fixation by means of an acetic-acid-alcohol mixture (1:3) has been found to give the best results. It is desirable to use a fixing reagent containing alcohol because it rapidly dissolves the chlorophyll with the

result that the leaf tissue becomes sufficiently transparent for microscopic examination. A period of 3½ to 4 hours is adequate for proper fixation.

After fixation, the fixing fluid is carefully decanted and replaced with a solution of lactophenol-alcohol. The lactophenol-alcohol solution is prepared according to the formula of Lepik[3] and is given below.

Phenol	10 g.	Glycerin	20 cc.
Lactic acid	10 cc.	Alcohol	20 cc.

In lactophenol-alcohol the leaf blade tissue becomes soft, pliable, and is easily manipulated. The material is next transferred to a clean dry slide, is flooded with 1% cotton blue (anilin blue W. S.) in 90% alcohol, and then covered with a cover slip.

In material prepared according to the above directions the dense cytoplasm of the germ tube and the mycelial growth within the tissues of the leaf blade take a deep blue stain, whereas the cells of the host remain colorless, or almost colorless (see Fig. 1, 2, and 3).

There are several advantages of this method over the ordinary paraffin method.

1. It eliminates the time and materials consumed in the long and tedious paraffin method.

2. It avoids the difficulty in studying the growth and penetration of the germinating conidia in sectioned material resulting from the frequent loss of critical stages during washing and dehydration.

3. It is possible to study the mycelium as a unit after the germ tube has entered the stoma and proceeded to ramify within the tissue of the host (see Fig. 1 and 3).

4. The relationship between cells of the host tissue and the parasite can be readily traced in whole mounts, whereas the picture has to be gathered more or less piecemeal in sectioned material.

The method outlined above has some objectionable features. The slides are temporary and cannot be retained in good condition, as a rule, for a period longer than a week. There is a gradual diffusion of the stain (anilin blue W. S.) into the host tissue with consequent loss of distinction between host and parasite.

A certain amount of care is required in transferring the leaf sections from one fluid to another as there is danger of washing off the fungus thru too vigorous manipulation of fluids or host tissue.

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[3]Lepik, E. 1928. Differential staining of Peronosporaceae. Phytopathology, 18, 869–72. 1928.

STUDIES ON HEMATOXYLIN STAINS[1]

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ABSTRACT.—Selected formulae and procedures for securing specific objectives in staining with hematoxylin are discussed in this article. The separate sections are numbered serially for ready reference, and the subject matter of the paper is arranged under the following self-explanatory divisions:

I.	Introduction	Sections 1–5
II.	Artificial ripening of hematoxylin	6–12
III.	Stock solutions of hematoxylin	13–17
IV.	Selection of a mordant	18–27
V.	Single-solution stains	28–30
VI.	Two-solution stains	31–37
VII.	Mordants for separate use	38–41
VIII.	Control of color	42–45
IX.	Control of density	46
X.	The problem of contrast	47–51
XI.	Low contrast stains	52–59
XII.	Medium contrast stains	60–63
XIII.	High contrast stains	64–67
XIV.	Summary	
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I. INTRODUCTION

1. The versatility of hematoxylin as a biological stain is attested by the remarkable way in which it has retained its leadership in competition with a host of newer, synthetic dyes. An excellent account of the history of hematoxylin staining was presented by Conn in 1929.

2. As soon as the ripening process, occurring spontaneously in hematoxylin solutions exposed to the air was recognized as an oxidative phenomenon (Mayer, 1891; Unna, 1892), investigations in this field sought the answer to a two-fold problem: (a) the production of

[1]It is a pleasure to acknowledge the capable assistance of Miss Margaret R. Hall, who has made many of the preparations, tested and rechecked formulae, and furnished independent judgements on such subjective aspects as density, contrast, and color of preparations.

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hematoxylin solutions which would be ready for use immediately after preparation, and (b) solutions having a long and uniform staining life. Many formulae have been developed. This paper presents the results of investigations carried on by the writer in a limited section of this two-fold field. These studies deal exclusively with normal animal tissues, and with general rather than special technics.

3. The rate of oxidation of hematoxylin is profoundly affected by its solvent. In neutral aqueous solutions hematoxylin may oxidize to form hematein in a few hours, more slowly in acid solutions, and more rapidly in alkaline solutions. In alcoholic solution, the rate is considerably slower. The addition of glycerin still further retards the rate of oxidation, an observation first made by Mayer (1896) and repeatedly verified since that time. The amounts of hematoxylin, hematein, and further oxidation products vary according to the age of the solution and the nature of the solvent. In all stages its composition, therefore, is very complex; its staining capacity is likewise variable.

4. The color of a hematoxylin solution changes progressively from water-white thru lilac, bright purple, deep purple, red, orange-red, orange-brown, to brown. (This color gamut holds only when no mordant is present.) At the purple stage the stain is most vigorous; the red stages are less so; the appearance of the orange-brown color marks the end of its useful life. The length of time required for a stain to reach the orange-red color is therefore an index of its useful life span. An unpublished series of experiments by the author illustrates this point. In one series of bottles, 10 ml. of water was placed in each; 10 ml. of 95% alcohol was placed in each of a second series. To each bottle was added 5 drops of freshly prepared stock 5% hematoxylin solution and one drop of NH_4OH. The aqueous stain reached the deep purple stage within one minute; the alcoholic stain required 39½ hours to reach a similar color. In the former the red-orange stage was reached in 18 hours; in the latter in 88 hours. The alcoholic solution then had a life five times greater than the aqueous solution.

5. When used with appropriate mordants, the color of the solution also indicates the color and nature of the staining of the tissue section. Following use of ammonium alum as a mordant, lilac and purple solutions stain nuclei blue; red solutions stain black; orange-red and orange-brown solutions stain both nuclei and cytoplasm a rusty brown color. The observation that iron hematoxylin stains nuclei blue after a short bath, and black after a prolonged bath is based not upon the time factor, but upon the oxidation factor which

the duration of the bath permits. It is entirely possible to secure preparations, after a brief treatment with the stain, which seem equivalent to those secured by prolonged use of Heidenhain's stain, (Cole, 1926). It can be demonstrated in a final histological preparation, that both the color and the selectivity of the stain depend upon a number of factors: (a) nature of the mordant, (b) the age (degree of ripeness) of the stain, and (c) the nature of the destaining agent.

II. Artificial Ripening of Hematoxylin

6. Many methods have been devised for rapidly ripening hematoxylin. Cuccati (1888), probably without recognizing the oxidizing effect of the chemical, reported that the use of KI in alum hematoxylin solutions gave a stable solution which never overstains. Since there is a possibility that Cuccati used a brown salt containing free iodine, his work was repeated by the writer, using pure KI, and the staining qualities checked against control solutions lacking that salt. Tests made immediately after preparation and again 50 days later clearly indicated that the pure KI used had not in any way affected the rate of oxidation of the hematoxylin solutions. It may be assumed, then, that the oxidation observed by Cuccati was due, not to the KI, but to free iodine existing in the salt. KIO_3 was used by Mayer (1903). The writer recently reported the use of iodine (Cole, 1941) as a ripening agent, alone or in combination with potassium permanganate.

7. H_2O_2 was used as a ripening agent independently by Mayer (1891) and Unna (1892), and was later reintroduced by Piazza (1912). Harris (1900) reported the value of HgO as a ripening agent, and a modification of his method was used by Galigher (1934) in his excellent stain. Camp (1931) ripened hematoxylin solutions by boiling them.

8. Anderson (1923) used chlorinated quicklime in ripening hematoxylin. Kohl and James (1931) and Neild (1934) used ultraviolet light in ripening Ehrlich's and Delafield's hematoxylins.

9. Hansen (1895) tried $KMnO_3$ as a ripening agent, for oxidizing mixtures of alum and hematoxylin. He assumed that this reagent would destroy all germs and make the solution keep better. It is still used in a few formulae, for example, in Mallory's phosphotungstic acid hematoxylin (1900, 1938), and deserves wider use.

10. Alkaline agents also are of value in ripening hematoxylin, but their use in single-solution stains is complicated by the speed with which they act and by the necessity of neutralizing them in order to avoid precipitation of the mordant in the staining solution. Von

Wistinghousen (1891) used $NaHCO_3$ for neutralizing stains, a process which involved some actual ripening.

11. Mayer (1891) dissolved hematoxylin crystals in dilute NH_4OH. The use of this compound as a ripening agent in some of the more recent formulae was discussed by Cole (1925, 1926). The value of another alkaline agent, Na_2HPO_4, was described by Cole (1933).

12. Kornhauser (1930) suggested the preparation of stains containing hematein instead of hematoxylin. This device, first used by Mayer (1891), does eliminate the necessity of preliminary oxidation, but it leaves unsolved the problem of controlling further oxidation.

Still other substitutes for hematoxylin were suggested by Proescher and Arkush (1928) who reported on the value of the metallic lakes of gallamin blue, gallocyanin and coelestin blue as nuclear stains. A part of their argument in favor of these stains was based on alleged inadequacies of hematoxylin itself, a point that Houseman (1928) was quick to challenge. The sum of all evidence appears to indicate that hematoxylin, when properly ripened, is still an excellent, perhaps indispensable, stain.

III. Stock Solutions of Hematoxylin

13. Stock solutions of hematoxylin may be either aqueous or alcoholic; the latter usually have a longer life. In any case they should be neutral or slightly acid, never alkaline, since alkalinity favors rapid oxidation.

Hance (1933a) added a small amount of $NaHCO_3$ to his stock solution of alcoholic hematoxylin, and stated that it had remained good for three years. That three years was the limit of its life became evident, when the same author (1933b) reported that the stock solution had deteriorated. Of course, any stock solution that keeps for three years needs no apology. In this case the longevity was apparently due to the presence of alcohol, and the high concentration of the hematoxylin.

Stock solutions should be stored in pyrex bottles rather than in bottles of the more easily soluble soda-lime glass. The following stock solution keeps for a considerable time, but is, of course, slowly oxidized.

Hematoxylin stock solution (unprotected)

95% alcohol	100 ml.
Hematoxylin crystals (certified)	2 g.

14. It has long been considered essential to have a well-ripened stock solution, but improved methods of oxidizing the stain rapidly

have now made this less important. It is frequently desirable to start with a completely unripened solution and to ripen it rapidly to the desired degree. Such a procedure gives more nearly uniform results than technics in which stock solutions of undetermined ripeness are used.

15. Acid sulphites such as $NaHSO_3$, $Na_2S_2O_4$ (hydrosulphite) and $K_2S_2O_5$ (metabisulphite) have been used in the preparation of non-oxidizing stock solutions. To limit the amount of bisulphite that goes into solution, Cole (1926) recommended the use of absolute alcohol, rather than 95% alcohol or water as a vehicle for the stock solution.

Hematoxylin stock solution (with metabisulphite)

Absolute alcohol	100 ml.
$K_2S_2O_5$	1 g.
Hematoxylin crystals (certified)	2 g.

It should be stored in a pyrex bottle, and shaken occasionally during the first day or two. The solution is straw-colored and will remain unoxidized for a long time. If the solution darkens, 3 or 4 drops of water should be added and the solution shaken thoroly. Aqueous dilutions of the stock solutions give better results if they are heated to boiling before incorporation in a staining solution.

16. An excess of $Na_2S_2O_4$ or $K_2S_2O_5$ is observed to damage the staining qualities of hematoxylin. This observation is in line with those of McClung (1923), who noted the injurious effect of the SO_2 formerly used as a bleach in the preparation of hematoxylin crystals. (Now the light-colored crystals are prepared commercially, not by bleaching, but by recrystallization—Conn, 1927).

17. Formalin is another reagent that protects hematoxylin against oxidation for a considerable period of time. The formula below was tested after a year's storage in a glass-stoppered pyrex bottle, and showed but slight signs of oxidation.

Hematoxylin stock solution (with formalin)

Formalin 1:20	100 ml.
Hematoxylin crystals (certified)	2 g.

To use either one of these protected stock solutions, one part of the stock solution should be diluted with three parts of water, and the mixture brought to a boil to expel the preservative. It is then ready for use in two-solution procedures (Section 31), or, combined with one and one half times the volume of saturated ammonium alum solution, will serve as an excellent single-solution stain (Sections 28–

30). For immediate use the stain may be ripened by the addition of 3 to 5 drops of 5% aqueous $KMnO_4$ per 100 cc. of ready stain.

IV. Selection of a Mordant

18. Altho successful staining with hematoxylin requires the use of a mordant, considerable latitude is available in both the kind of a mordant and the method of using it. The simplest but also the least adaptable method is one in which mordant and stain are combined in a single solution. Such solutions must be more or less acid to prevent formation of a precipitate which will weaken or destroy their staining capacity.

19. If a long-lived solution is desired, mordants having little or no oxidizing action must be used, or an oxidation-reduction buffer must be included. Ammonium alum, potassium alum, phosphomolybdic acid, phosphotungstic acid, and the iron-alum-ferrous-sulfate mordant proposed by Earle (1939) are representative.

20. If a long life is not a requisite, then mordants having a vigorous oxidizing effect can be used. Such solutions should be prepared immediately before use, and must be discarded afterward, since their period of usefulness is often a matter of hours. Ferric chloride, ferric acetate, ferric alum, and mordants containing permanganate or chromium salts are of this type. Intense and rapid staining with varying degrees of contrast are readily secured.

21. The use of mordant and stain as separate solutions introduces additional steps in the procedure of staining, but offers far greater variety and better control of the results. It is essential that excess mordant be removed from the sections before they are placed in the hematoxylin bath. Such combinations as an acid mordant followed by an alkaline solution of hematoxylin may be used, i.e. reagents which could not be successfully combined in a single solution.

22. Heidenhain's (1892) original formula called for 1.5 to 4.0% aqueous iron alum. Lang (1936) found that the addition of acetic and sulfuric acids gave added stability to the iron alum solution. Similar results were secured by Earle (1939) thru the use of equal parts of ferrous and ferric alum in place of the usual ferric alum in Janssen's stain (Janssen, 1897).

23. Mallory (1900) used a 10% aqueous $FeCl_3$ solution as a mordant, stained tissues in 1% aqueous hematoxylin, and destained in 0.25% aqueous $FeCl_3$. Häggqvist (1933) used weaker solutions (3–5%), followed by a bath in a freshly prepared hematoxylin solution. He noted that nuclei were stained blue, and that destaining or differentiation was easier than with Heidenhain's iron hematoxylin. Weigert (1904) added HCl to a $FeCl_3$ mordant to secure preparations

of high contrast. The present writer has observed that the addition of 5 to 10% citric acid to a $FeCl_3$ mordant gives an excellent high contrast picture. (Section 67).

24. When NH_4OH is added to a solution of $FeCl_3$ in quantities sufficient to cause a distinct darkening, the mordanting effects are greatly altered. Both nuclei and cytoplasm stain to give a preparation of low contrast. (Section 41).

25. Ferric acetate permits a very heavy deposit of stain in both the nuclei and the ctyoplasm. (Section 55). It was used by Bütschli (1892) for staining sections of protozoa. When freshly prepared this mordant has the color of tincture of iodine, but after a few days a muddy precipitate of $Fe(OH)_3$ may be formed. This precipitate may, however, be dissolved by the addition of $FeCl_3$ solution.

26. The following mordant was reported by Cole (1926).

50% alcohol	20 ml.
Ferric chloride	1 g.
Glacial acetic acid	2 ml.

It was followed by ammonia-ripened hematoxylin. (Section 36). Freshly prepared, the mordant gives a fairly sharp nuclear stain, but as the acetic acid and alcohol combine, the stain becomes somewhat less selective for nuclei. Knower (1939) reported a modification of this formula useful for staining thick sections of spinal cord and brain stem. Its use in staining plant chromosomes was reported in Lee's Vade Mecum (1937).

27. Chromic acid used alone is not satisfactory as a mordant, but, combined with alums or with ferric salts, it increases the intensity of staining. The stain is not limited to the nuclei, but is also heavy in the cytoplasm. If excess mordant is not carefully removed by washing, the sections will stain unevenly or fade. Hansen (1895) noted that the addition of CrO_3 or $K_2Cr_2O_7$ gave a darker tone to the staining results. This effect was clearly due to further oxidation of the hematoxylin. Sections stained after treatment with mordants containing chromium salts are extremely sensitive to acid baths. If destaining or differentiation is attempted, the usual 0.2% HCl should be diluted to about 1/10 of its concentration, and the process carefully watched to avoid excessive loss of stain. After destaining is completed, the sections should be thoroly washed.

V. Single-Solution Stains

(*Mordant and stain combined*)

28. Phosphomolybdic-acid hematoxylin (Mallory 1891), phosphotungstic-acid hematoxylin (Mallory 1900), and ferric-alum-

ferrous-sulfate hematoxylin (Earle 1939) are representative of a number of stains with excellent keeping qualities. Many long-lived single-solution stains consist essentially of a solution of hematoxylin mixed with a solution of ammonium (or potassium) alum. The degree of ripeness of the hematoxylin, the relative proportion of dye and mordant, and the acidity of the solution affect the results secured.

29. Iodine-ripened hematoxylin. This formula more nearly approaches the ideal than any known to the author. It is simple to prepare, gives maximum staining at once, has high contrast and remains practically unchanged for a long time. The concentration of hematoxylin (0.5 g. to the liter) is low, and may be increased to a total of 2 g. per liter if desired, with a corresponding increase in density and a slight loss in contrast. The method of preparation is:

Dissolve 0.5 g. hematoxylin crystals in 250 ml. of warm water.
Add 50 ml. of 1% iodine (in 95% alcohol).
Add 700 ml. of saturated aqueous ammonium alum solution.
Bring this mixture to a boil, and allow it to cool. It is then ready for use.

One year after preparation, this stain gave essentially the same results as it gave as a fresh stain. Any slight tendency for the cytoplasm to stain can easily be corrected by the addition of small amounts of alum solution.

30. Permanganate-ripened hematoxylin. The solution described above may be brought immediately into heavier staining power by the addition of 2–5 drops of 5% $KMnO_4$, or a separate solution may be prepared as follows:

Hematoxylin crystals	1 g.
Warm distilled water	200 ml.
5% $KMnO_4$	2 or 3 ml.
Saturated aqueous ammonium alum	800 ml.

This stain has less contrast than the iodine-ripened hematoxylin, and is especially useful where staining of cytoplasmic details as well as heavy nuclear staining are desired.

VI. Two-Solution Stains

(Separate mordant and stain)

31. Two-solution methods are usually not well adapted to the quantity production of slides, but are unexcelled where the operator wishes to check the development of each slide individually. Coplin

jars may be used for the mordant and stain if a large number of slides are to be run thru at one sitting. Generally, however, it is more economical to use a staining board on which each slide is covered with a puddle of the reagents in sequence. This method has the advantage of using small amounts of reagents and of facilitating atmospheric oxidation of the stain.

32. In the further interests of economy, special dropping bottles may be used. These bottles permit the operator to place small amounts of the reagents directly upon the sections to be treated.

33. A mordant of any kind may be used if it is followed by treatment with a well-ripened hematoxylin stain, such as ammonia-ripened stain, (Section 36), or phosphate-ripened stain, (Section 37), or Heidenhain's hematoxylin solution. If, however, one wishes to use a relatively unripened stain, the mordant should include substances of considerable oxidizing power, such as ferric or chromium salts. One value of the two-solution method is that the stain can be preceded by mordants which could not be used in combination with the stain. For example, sections mordanted in 5% aqueous $FeCl_3$ may, after rinsing, be stained in ammonia-ripened hematoxylin. If an attempt were made to combine these two solutions, a heavy precipitate of $Fe(OH)_3$ would be thrown down. In using any mordant it is essential to wash out the excess before placing the sections in the hematoxylin solution. Otherwise the staining will be spotty and irregular.

34. A variant of the two-solution method is the process of double mordanting. Here treatment with the mordant is followed by treatment with a single-solution stain (which, of course, contains both a mordant and the hematoxylin). A wide variety of effects may be secured. If the premordant is ammonium or potassium alum, the single-solution (combination) stain following it must be well ripened to give satisfactory results. Iodine-ripened hematoxylin and permanganate-ripened hematoxylin solutions are suitable.

On the other hand, premordants such as ferric or chromic salts, which have a high oxidizing power, may be followed by a freshly prepared and unripened combination solution, with excellent, high-contrast results. This method appears to be one of the best ways of using an unripened stain. Furthermore, the minute amounts of mordant carried over into the stain speed up oxidation, so that the single-solution stain soon becomes sufficiently ripened to permit its use alone.

35. Some reagents used in two-solution procedures are alkaline and tend to cause sections to become detached from the slide. This

difficulty can be avoided by the use of a celluloid (or celloidin) film,[3] followed by an alcohol-chloroform (9:1) bath. Of course, in any method where wet slides are to be studied, it is advisable to put a glass plate on the microscope stage and use only the low power objective for examination.

36. Ammonia-ripened hematoxylin. (Note that this solution contains no mordant and must therefore be preceded by a mordant bath.)

To 5 ml. water in a dropping bottle add 5 drops of stock hematoxylin (Sections 13–17) and one drop of NH_4OH.

The stain is ready for use within 30 seconds and has a life of from 4 to 6 hours. If the 5 drops of stock hematoxylin are added to 10 drops of water and a drop of NH_4OH added, oxidation proceeds rapidly. After a minute, 5 ml. of 95% alcohol may be added. The stain is ready for immediate use and has a life of about 24 hours. The substitution of 95% alcohol for the water in the preparation of the ready stain gives a solution ready for use in about 20 minutes and having a life of three or four days. All of these modifications have a rich purple color, indicating vigorous staining power. Each runs thru a progressive oxidation, and the color of the solution at any point is a clue to the color of the final preparation (Section 4). The mordants which can be used with this stain are listed in Sections 38–41.

37. Phosphate-ripened hematoxylin. (Note that this solution contains no mordant, and must therefore be preceded by a mordant bath). To prepare a ready solution: to 5 ml. water in a dropping bottle, add 10 drops of stock hematoxylin solution (Sections 13–17) and either (a) a small crystal of Na_2HPO_4 (NaH_2PO_4 or Na_3PO_4 will not serve) or (b) 4 drops of a 10% aqueous solution of the phosphate. The mixture begins to turn pink within a few seconds, and after two or three minutes becomes an intense clear, cherry red; at this point the stain is ready for use. Its life is about 24 hours. Mordants suitable for use with this stain are listed in Sections 38–41.

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[3]Celluloid solution. A stock solution consists of 5 g. of clear celluloid (scrap photographic film base may be used) dissolved in 20 ml. of glacial acetic acid, and then diluted with absolute ethyl alcohol to make a total volume of 100 ml. To make the ready solution, 10 ml. of this stock solution is added to 90 ml. of absolute alcohol. This makes a ready solution about as strong as can be safely used. Much weaker solutions will often give surprising protection to sections. The film forms a meshwork over the sections, and interferes but slightly, if at all, with the penetration of the mordant and the stain.

VII. Mordants for Separate Use

The mordants described below were used in 5% aqueous solution. The amounts of any modifying ingredients added were based upon a total volume of 100 ml. of mordant solution. The color values given represent those secured by minimal (10 minutes) staining.

38. Ammonium or potassium alum will not serve with unripened hematoxylin solutions, but gives clear, blue, high-contrast results when used with ripened single-solution stains (Sections 29, 30) or with rapidly ripened hematoxylin solutions (Sections 36, 37).

Because of their vigorous oxidizing action, the mordants given below cannot be used without buffers, in long-lived, combination solutions. They are useful, however, in short-lived, combination solutions, and in two-solution stains.

39. Ferric ammonium alum alone gives a blue color, medium contrast and medium density. When 2 or 3 drops of HCl are added the contrast is increased and the density slightly lowered.

40. $FeCl_3$ alone gives a blue color, medium contrast and medium density. When HCl is added (Weigert, 1904) the contrast is markedly increased. Similarly the addition of 5 g. of citric acid gives greatly increased contrast, and this feature is retained during the life of the stain. When 0.1 g. of CrO_3 is added to $FeCl_3$ mordant the color becomes blue-black, the contrast becomes medium and the density is greatly increased.

41. Two other modifications of $FeCl_3$ mordant have proved useful. The addition of 20 ml. of glycerin, followed by NH_4OH, drop by drop, until the solution darkens to resemble an iodine solution, gives a preparation having a blue-black color, low contrast and medium density. The addition of 5 g. sodium acetate to $FeCl_3$ mordant gives closely similar results.

VIII. Control of Color

42. The color of the finished sections depends upon several factors, including: (a) the nature of the mordant; (b) the nature, age, and degree of oxidation of the staining solution; (c) the duration of staining; and (d) the use of destaining or after-bath solutions.

43. Ammonium alum forms the basis for a bright blue nuclear stain when followed by hematoxylin. Potassium alum, followed by hematoxylin, gives a lilac or violet stain, somewhat more transparent than ammonium alum. Chrome alum, followed by hematoxylin gives a cold gray-blue color, denser than that secured with either of the other alums. Iron alum gives a blue to black stain when followed by hematoxylin. The oxidizing action of the last two mordants

is fairly rapid; they cannot therefore be used without buffering in a single-solution stain, if longevity is desired.

44. The usual nuclear color of single-solution stains is blue or purplish blue. These colors are characteristic of fresh stains, or of older stains where the duration of the staining is brief. The production of blue color in finished sections is favored by: (a) the use of ammonium or potassium alums as mordants, (b) a relatively fresh (that is, unoxidized) stain, (c) brief duration of staining, and (d) the use of 0.2% solutions of HCl or citric acid as destaining agents.

45. On the other hand, the production of black results in finished preparations is favored by: (a) the use of $FeCl_3$, ferric alum, ferric acetate, or chrome alum as mordants, followed by (b) somewhat prolonged treatment in well-ripened hematoxylin, (c) destaining in dilute HNO_3 or in diluted mordants, followed by thoro rinsing, and (d) immersion in a bath of 1% aqueous $K_2Cr_2O_7$. After this last bath the slide should not be exposed to an acid solution, for the least trace will destroy the stain. Excess dichromate should be removed by thoro washing. If single solutions of the ammonium-alum-hematoxylin type are used, they should be preceded by an oxidizing mordant, such as $FeCl_3$. If slides are treated on a staining board, a drop of dilute (ca. 1:5) NH_4OH, 0.1% Cr_2O_3, or 0.1% $KMnO_4$ may be added directly to the stain puddle and mixed by rocking the slide. These reagents will accelerate oxidation. Of course, it is seldom feasible to use all of the procedures suggested in the treatment of any one slide. The factors favoring blue or black staining appear to depend upon a slight or a considerable oxidation respectively of the hematoxylin.

IX. Control of Density

46. The density of a slide is related to its color in that extreme density appears to go along with a tendency toward blue-black color. As previously mentioned, chromic acid alone is not a satisfactory mordant, but if it is added in small amounts to any mordant, it enormously increases the density. Ripe stains also tend to give greater density than fresh stains for a given time. Prolonged staining greatly increases the density of the stain. The chief objective is to secure a sufficient amount of stain in the tissues so that subsequent treatment will give the desired contrast between the darkest and the lightest elements of the tissue.

X. The Problem of Contrast

47. The question of contrast is of paramount importance. Some problems demand a low degree of contrast in order that both nuclear

and cytoplasmic details may be delineated. Others require staining limited to the nucleus. This can often be achieved by suitable destaining, but it is easily possible to prepare stains which give maximum contrast without the need of a destaining bath.

48. That the pH of the solution used in the staining process profoundly effects the results has been clearly described in an admirable article by Craig and Wilson (1937). It has long been recognized that moderately acid solutions favor nuclear staining (Ehrlich, 1886). The various alums give acid solutions upon dissociation, but the degree of acidity is usually such that both nucleus and cytoplasm are stained; that is, there is medium contrast. By adding acetic acid, Ehrlich produced a stain that had high contrast; the nuclei were heavily stained but the cytoplasm was scarcely colored. As more acid is added the differentiation between nucleus and cytoplasm increases. Excessive amounts of acid reduce the density of staining in both nucleus and cytoplasm, until a point is reached where little if any staining occurs.

49. The opposite effect can be produced by bringing the pH of the solution nearer the neutral point. For example, small amounts of sodium acetate added to an alum-hematoxylin solution cause the stain to color both cytoplasm and nuclei, with a resultant low contrast. Such methods should be used with caution however, for if the solution is brought too close to neutrality, an abundant precipitate of $Al(OH)_3$ will be thrown down and the stain rendered useless until the precipitate is redissolved by the addition of acid.

50. In two-solution procedures, also, the pH of the mordant is an important factor in determining contrast. A slide mordanted in ferric acetate and subsequently stained in phosphate hematoxylin gives a dense, low-contrast picture. With $FeCl_3$ as a mordant, the stain gives a medium contrast. If hydrochloric, acetic or citric acid is added to the $FeCl_3$ mordant, subsequent staining gives high contrast. The high contrast secured by Lang (1936) was due to the addition of acetic and sulfuric acids to the ferric alum used as a mordant.

51. Destaining may also be used to increase contrast. The effects of the process depend not only upon the nature of the original mordant, but also upon the destaining agent used. Dilute solutions of acetic or hydrochloric acid, ammonium or potassium alum apparently merely dissolve the lake; CrO_3, HNO_3, $FeCl_3$ and iron alum not only dissolve the lake but oxidize it. Thus the destaining action may achieve not only an increase in differentiation, but also a change in the color of the preparation. Zimmerman (1893) and Tuan (1930)

are among those who used aqueous picric acid to secure this two-fold effect on plant tissues. Hutner (1934) used picric acid and H_2O_2 on protozoa, with similar results. Picric acid forms an essential part of Masson's trichrome stain for connective tissue (Masson 1928).

XI. Low Contrast Stains

52. Low contrast stains color the cytoplasm nearly as heavily as they do the nuclear material. The nuclei appear as more or less homogeneous bodies, and the cytoplasmic structure is clearly marked. Counterstaining is relatively unimportant, since most of the cytoplasm has been preempted by the hematoxylin. Sections are usually stained gray, blue, or blue-black. Muscle, nerve, connective and other tissues stain in slightly different shades. Low contrast stains are of value in marking the general boundaries of tissues and organs. They may, for that reason, be called topographical stains.

53. The following low contrast procedures have been found useful. Each followed fixation in Bouin's picro-formal-acetic solution. The stain used was phosphate-hematoxylin (Section 37), applied from a dropping bottle to slides resting on a staining board.

54. Mordant: 5% ferric alum, 10 min.
Stain: 5 min. or less.
Destain: 0.2% HCl, 2 to 10 sec.
Characteristics: blue, topographical.

55. Mordant: ($FeCl_3$, 10 g.; sodium acetate, 10 g.; water, 100 ml.) 10 min.
Stain: 10 min.
Destain: none.
Characteristics: blue-black, topographical.

56. Use celluloid film.
Mordant: 5% $FeCl_3$, 10 min.
Stain: Add one drop conc. NH_4OH directly to the stain on the slide. As soon as the pool of stain becomes orange-red, rinse the slide and finish it.
Characteristics: black, topographical.

57. Mordant: ($FeCl_3$, 5 g.; water 100 ml.; CrO_3, 0.1 g.) 10 min.
Stain: 10 min.
Destain: 0.2% acetic acid, 1 min.
Characteristics: dense, black, topographical.

58. Mordant: 5% $FeCl_3$, 10 min.
Stain: 10 min.
Destain: (50% alcohol, 100 ml.; $FeCl_3$, 0.1 g.; conc. HCl, 0.1 cc.) 3 min.
Characteristics: dense, black, topographical.

59. Mordant: (10% $FeCl_3$, 10 ml.; 1% CrO_3, 2 ml.) 10 min.
Stain: 10 min.
Destain: 0.2% HCl, 1 min.
Characteristics: dense, black, topographical.

XII. Medium Contrast Stains

60. Most hematoxylin stains fall into this group. Prolonged staining of any of the low contrast stains, together with adequate destaining will give medium contrast. Among the single-solution stains permanganate-ripened hematoxylin (Section 30) has given excellent results. In the following two-solution procedures, phosphate hematoxylin (Section 37) was used.

61. Mordant: 5% $FeCl_3$, 10 min.
Stain: 10 min.
Destain: 0.2% HCl or HNO_3.
Characteristics: medium density, nuclei blue-black.

62. Mordant: (5% $FeCl_3$, 50 ml.; concentrated HCl, 2 drops) 10 min.
Stain: 10 min.
Destain: 0.2% HCl, 10 sec.
Characteristics: medium density, nuclei blue-black.

63. Mordant: (50% alcohol, 100 ml.; $FeCl_3$, 5 g.; citric acid, 1 g.) 10 min.
Stain: 10 min.
Destain: none.
Characteristics: medium density, nuclei blue-black.

XIII. High Contrast Stains

64. In the great majority of cases the user of hematoxylin desires a selective nuclear stain. This can often be secured regressively by overstaining the sections and then destaining the cytoplasm by treatment with dilute mordant, acetic acid, HCl, or combinations of dilute mordant and dilute acids. HNO_3 has been but little used as a destaining agent, altho it appears to be an excellent reagent when used in concentrations varying from 0.1 to 0.3%. Not only does it destain the cytoplasm, but it oxidizes the stain remaining in the nuclei, so that the color changes from blue to a rich black.

65. High contrast can also be obtained by adding acid substances to single-solution stains. Thus Ehrlich used acetic acid to increase nuclear differentiation. Nuclear selectivity is secured in Delafield, Harris, and Galigher stains by the addition of more saturated ammonium alum, as soon as the solution shows signs of over-staining.

The writer has obtained excellent results by adding citric acid to permanganate-ripened stains (Section 30). By varying the amounts of this acid from 1 to 10% one can secure reagents which stain nuclei heavily and cytoplasm lightly; stain nuclei heavily and leave cytoplasm unstained; or stain only chromatin granules or the chromosomes. Reference has already been made to iodine-ripened hematoxylin (Section 29), a single-solution stain of high contrast.

The following two-solution procedures were used in connection with phosphate hematoxylin, (Section 37), and gave excellent results.

66. Mordant: (saturated ammonium alum, 50 ml.; citric acid, 5 to 10 g.; water, 50 ml.) 10 min.
    Stain: 10 min.
    Destain: none.
    Characteristics: nuclei blue; cytoplasm faint or colorless.
67. Mordant: (water, 100 ml.; $FeCl_3$, 5 g.; citric acid, 5 to 10 g.) 10 min.
    Stain: 10 to 20 min.
    Destain: none.
    Characteristics: nuclei, blue-black; cytoplasm, faint or colorless.

XIV. Summary

1. The composition of a number of hematoxylin solutions is discussed and the effects of their different components are considered.
2. The ripening of hematoxylin, and the problem of securing long-lived staining solutions are discussed.
3. The effects of different mordants on longevity, color, density and contrast are considered.
4. The merits of single solution and two-solution stains are analyzed.
5. A number of tested formulae and procedures are presented.

XV. LITERATURE CITED

Anderson, J. 1923. A rapid method of ripening haematoxylin with hypochlorite. J. Path. and Bact., **26**, 303.

Bütschli, O. 1892. Untersuchung über mikroskopische Schäume und das Protoplasma. Zts. wiss. Mikr., **9**, 189–98.

Camp, W. H. 1931. Rapidly ripened hematoxylin and its uses. Science, **74**, 661–2.

Cole, Elbert C. 1925. A rapid iron-hematoxylin method. Anat. Rec., **34**, 328 (Abstract).

————. 1926. A rapid iron-hematoxylin technique. Science, **64**, 452–3.

————. 1928. Blue or black stains with hematoxylin. Anat. Rec., **41**, 63 (Abstract).

————. 1933a. A rapid hematoxylin technique for routine use. Anat. Rec., **57**, 105 (Abstract).

————. 1933b. The ripening of hematoxylin. Anat. Rec., **57**, 106 (Abstract).
————. 1941. A Text-book of Comparative Histology. The Blakiston Co., Philadelphia.
CONN, H. J. 1927. The hematoxylin problem. Stain Techn., **2**, 1–3.
————. 1929. The history of staining. Logwood dyes. Stain Techn., **4**, 37–48.
CRAIG, RODERICK, and WILSON, CHARLES. 1937. The use of buffered solutions in staining: theory and practice. Stain Techn., **12**, 99–109.
CUCCATI, GIOVANNI. 1888. Sopra una soluzione alcoolica di ematossilina. Zts. wiss. Mikr., **5**, 55–6.
EARLE, W. R. 1939. Iron hematoxylin stain containing high concentration of ferrous iron. Science, **89**, 323–4.
EHRLICH, P. 1886. Hämatoxylinlösung. Zts. wiss. Mikr., **3**, 150.
GALIGHER, A. E. 1934. The Essentials of Practical Microtechnic in Animal Biology. A. E. Galigher, Inc., Berkeley, Cal.
HÄGGQVIST, G. 1933. Eisenchloridhämatoxylin. Zts. wiss. Mikr., **50**, 77–82.
HANCE, R. T. 1933a. Improving the staining action of iron hematoxylin. Science, **77**, 287.
————. 1933b. A note on iron hematoxylin. Stain Techn., **8**, 117.
HANSEN, H. J. 1895. Eine schnelle Methode zur Herstellung des Böhmer'schen Hämatoxylins. Zool. Anz., **18**, 158–60.
HARRIS, H. F. 1900. On the rapid conversion of haematoxylin into haematein in staining reactions. J. Appl. Micr., **3**, 777–80.
HEIDENHAIN, M. 1892. Über Kern und Protoplasma. Zts. wiss. Mikr., **9**, 198–206.
HOUSEMAN, PERCY A. 1928. Discussion of paper on "Metallic lakes of the oxazines as nuclear stain substitutes for hematoxylin". Stain Techn., **3**, 70.
HUTNER, S. H. 1934. Destaining agents for iron hematoxylin. Stain Techn., **9**, 57–9.
JANSSEN. 1897. Hématoxyline noire. La Cellule, **14**, 207. Quoted from Lee (1937).
KNOWER, H. McE. 1930. A rapid method for staining sections of the spinal cord and brain-stem. Science, **72**, 172–3.
KOHL, E. J., and JAMES, C. M. 1931. A method for ripening hematoxylin solutions rapidly. Science, **74**, 247.
KORNHOUSER, S. I. 1930. Hematein—its advantages for general laboratory work. Stain Techn., **5**, 13–5.
LANG, A.G. 1936. A stable high-contrast mordant for hematoxylin. Stain Techn., **11**, 149–51.
LEE, A. B. 1937. The Microtomist's Vade Mecum, *10th ed.* The Blakiston Co., Philadelphia.
MALLORY, F. B. 1891. Phospho-molybdic acid haematoxylin. Anat. Anz., **6**, 375–6.
————. 1897. On certain improvements in histological technique. J. Exp. Med., **2**, 529–33.
————. 1900. A contribution to staining methods. J. Exp. Med., **5**, 15–20.
————. 1938. Pathological Technique. W. B. Saunders Co., Philadelphia.
MASSON, P. 1928. Carcinoids (argentaffin-cell tumors) and nerve hyperplasia of the appendicular mucosa. Amer. J. Path., **4**, 181–212.
MAYER, PAUL. 1891. Über das Färben mit Hämatoxylin. Mitt. Zool. Stat. Neapel, **10**, 170–86.
————. 1896. Über Schleimfärbung. Mitt. Zool. Stat. Neapel, **12**, 303–30.

————. 1903. (Title not available.) Zts. wiss. Mikr., **20**, 409. Quoted from Lee (1937).

McCLUNG, C. E. 1923. Haematoxylin. Science, **58**, 515.

NEILD, H. W. 1934. A rapid method for the preparation of Delafield's haematoxylin. Science, **79**, 209–10.

PIAZZA, CESARE. 1912. L'invecchiamento rapido delle soluzioni ematossiliniche. Zts. wiss. Mikr., **29**, 69–71.

PROESCHER, F., and ARKUSH, A. S. 1928. Metallic lakes of the oxazines (gallamin blue, gallocyanin and coelestin blue) as nuclear stain substitutes for hematoxylin. Stain Techn., **3**, 28–38.

SASS, J. E. 1929. A modification of Mayer's hematoxylin. Stain Techn., **4**, 127–9.

TUAN, H. C. 1930. Picric acid as a destaining agent for iron hematoxylin. Stain Techn., **5**, 135–8.

UNNA, P. G. 1892. Über die Reifung unseres Färbstoffe. Zts. wiss. Mikr., **8**, 475–87.

VON WISTINGHAUSEN, C. Untersuchungen über die Entwicklung von Nereis Dumerilii. Mitt. Zool. Stat. Neapel, **10**, 41–74.

WEIGERT, C. 1904. Eine kleine Verbesserung der Hämatoxylin- van Gieson-Methode. Zts. wiss. Mikr., **21**, 1–5.

ZIMMERMAN, A. 1893. Botanical Technique. Translated by J. E. Humphrey. Henry Holt and Co., New York. (Quoted from Tuan 1930).

NOTES ON TECHNIC

Method for the Permanent Mounting of Sections Stained for Fat

Tissue fixed in formol saline is placed in a thick gum acacia solution in distilled water for 24 hours. Sections are cut by the freezing method at the desired thickness and are then treated in the following manner:

1. Stain with Herxheimer's Sudan IV solution and hematoxylin, or with other stains for fat which might be preferred such as osmic acid, Sudan black B and Nile blue sulphate.
2. After the staining procedures have been carried out, place the sections in distilled water.
3. Draw each section onto a slide from the distilled water and drain off excess distilled water.
4. Cover each section with a few drops of a diluted solution of gum acacia (10% in distilled water).
5. Allow the excess of gum acacia to drain off well, each slide being left in a vertical position, and allowed to dry at room temperature.
6. When the sections and slides are perfectly dry, place in xylene for just sufficient time to clear the sections—not more than 15 seconds. Then mount with Canada balsam or gum dammar as for paraffin sections.

The above method gives clear finished sections and far greater permanency than by mounting in either Farrant's medium or glycerin jelly.—T. N. Salthouse, *Department of Pathology, Prince of Wales' General Hospital, London, England.*

Recovery of Xylene

In recovering xylene by re-distillation in the laboratory, the process is greatly facilitated by passing a gentle air stream thru the flask over the xylene and out thru the condenser.—R. D. Lillie, *Division of Pathology, National Institute of Health, Bethesda, Md.*

Handling Extremely Thin Paraffin Sections

Paraffin sections 2–4 μ in thickness are often difficult to handle as they come off the microtome knife because of drafts, electrostatic forces, or heat. The following procedure eliminates these difficulties.

On the face of the microtome knife opposite the part of the edge to be used a pocket is built of fairly firm grease such as stearine (an animal by-product). To save grease, to prevent the pocket from gradually sliding down from the edge of the knife, and to facilitate moving of the pocket, a small frame of elder pith[1] or of balsa wood can be constructed. This pocket is then filled with cool water until the "lake" formed just bulges above the keen edge of the microtome knife. As the sections are cut they float out over the surface of the water where they may easily be picked up with a probe and transferred to the drop of water on a microscope slide.

As the various portions of the knife dull, the pocket and lake can be moved to another position with little manipulation. The thin sections are readily laid flat on the microscope slide.—E. Adelhelm, *1568 Washington St., Denver, Colo.*

[1]Suggestion of Dr. S. I. Kornhauser and Mr. Webster of Univ. of Louisville Medical School.

LABORATORY HINTS
FROM THE LITERATURE

A DEPARTMENT DEVOTED TO ABSTRACTS OF BOOKS AND PAPERS FROM OTHER JOURNALS DEALING WITH STAINS AND MICROSCOPIC TECHNIC IN GENERAL

MICROTECHNIC IN GENERAL

DAVEY, R. J. **Preparing and mounting rock sections.** *The Microscope*, 4, 255–7. 1941.

The few sections of hard rocks required by the average microscopist can be prepared by hand-working methods, such as the following: Cut the rocks to a suitable size with hammer and chisel, the attempt being to obtain even slices. Select two pieces of similar size; using water for a lubricant, grind a flat surface on each with a broken piece of carborundum wheel or with a worn-down grinding wheel. Do not use the side of a fast-running wheel. To secure a finer face, use a disc or a rectangular block of cast iron of about 6 in. diameter. On the disc or plate use first No. 100 emery powder with water, moderate pressure, and a circular motion; follow with No. 120, then with No. 150 powder. (A flat surface, but not a high polish, is essential. An apparent curvature may be corrected, and scratches may be reduced with a piece of Water of Ayr stone.) Rub finally on a piece of No. 00 emery or on fine garnet paper. Fasten the two specimens, smoothed side down, with hard Canada balsam in the center of a glass plate about 3½ × 2½ in., to whose opposite side is attached a glass knob. When the balsam is hard, grind away the rough side of the specimens, using the same grinding process as before but with great care. When the section begins to become transparent, finish the grinding with putty powder or a plate of flat glass or with a piece of the finest garnet paper laid on the glass plate. (Scratches may again be removed with the Water of Ayr stone.) Thoroly wash and dry; place the sections, covered with benzene, in a dust-proof glass container. After an hour or so, lift the plate with forceps, place it beside a glass slip on a flat surface, and with a needle, slide each section off the plate and into its place on the glass slip. Place a drop of the mountant on the section and apply a cover glass. Then place the slip in an oven that is warm but not hot enough to boil the mounting medium. The method, with some modifications, may be used with soft sedimentary rocks, laminated rocks, bones, teeth, and the stones of fruits.—*C. E. Allen.*

FRENCH, A. J. **Deep Cells.** *The Microscope*, 5, 81. 1942.

Cells are made of 7/8 in. squares cut with a fine saw from sheet celluloid about 1.5 mm. thick—the material used for set-squares. A ½ in. hole is drilled in the center of each square; the edges of the hole and of the square are smoothed with fine glass-paper. The polish already present on the flat surfaces is rubbed with glass-paper to obtain a smooth matt. One surface of each square is evenly coated with gold size (preferably old) and cemented centrally on a 3 × 1 in. glass slide. The cell is allowed to dry slowly for several weeks.

Such cells are suitable for either aqueous or resinous media. Over a number of years the celluloid has not warped nor separated from the slides.—*C. E. Allen.*

KENDALL, R **Quantitative microscopy.** *The Microscope*, 4, 258–9. 1941.

The method described consists in mixing weighed quantities of the substance to be examined and of the spores of *Lycopodium clavatum* L. By examining the mixture under the microscope and counting fields, the proportion of spores to any countable feature can be determined, and hence the percentage of the particles in the original substance. The spores are remarkably uniform in size, averaging 94,000 per milligram. Since their appearance is unaltered by boiling in dilute acids and alkalies, they can be added to any powder before treatment. Intimate mixture is secured by weighing out the powders on a glass plate and mixing them

a little at a time with the agent with which they are to be treated. For study, the particles must be suspended in some medium, such as glycerin, gum tragacanth, olive oil, or castor oil.—*C. E. Allen.*

KIRKPATRICK, J., and LENDRUM, A. C. **Polystyrene resins for mounting.** *The Microscope*, **4**, 210. 1940.

Mounts prepared, as described in a previous paper (*J. Path. and Bact.*, **49**, 592), with a simple xylene solution of a synthetic resin, a polystyrene, show marked retraction under the cover slip after a few weeks. It is found that the retraction can be prevented by the addition of a plasticizer. That used, tricresylphosphate, tho one of the cheapest, is probably not the most stable. Studies are being made of the value of other substances with plasticizing power, such as dibutyl phthalate. Unlike Canada balsam, the polystyrene is insoluble in a mixture of alcohol and xylene. Hence in clearing sections the absolute alcohol must be completely replaced by xylene.—*C. E. Allen.*

SPENCE, D. S. **Notes on mounting V.** *The Microscope*, **4**, 197–201. 1940.

Cajeput oil (also spelled "cajuput") has been reported to clear better than clove oil, and is certainly cheaper. It is obtained from *Melaleuca leucadendron* L., a tree of northern Australia and the Malay Archipelago. Its refractive index is 1.462 to 1.472.

Solutions of hydrargiodides have been long known, but little used for mounting. First introduced was potassium hydrargiodide, made by saturating water with KI and HgI_2. Similar media can be made by saturating glycerol or water with NaI or BaI_2 together with HgI_2. Statements are conflicting as to refractive indices, tho these are high. All have high specific gravities and are very sensitive to moisture, precipitating HgI_2, but can be easily sealed with soft paraffin. They are excellent preservatives, and lower refractive indices can be obtained by using lesser concentrations.—*C. E. Allen.*

SPENCE, D. S. **Notes on mounting VI.** *The Microscope*, **4**, 230–8. 1941.

The softening point of the polystyrene used in mounting is higher than that of Canada balsam, so that objects to be mounted in it require a specially heated hot plate. Its solution in xylene passes rapidly from a viscid to a coherent state. It will not take objects from liquefied phenol or from phenol-chloral-hydrate, in which liquids it is insoluble. It takes longer than Canada balsam to soak into thick objects. Of liquids tried, paraldehyde, a polymeride of acetaldehyde, has been found in most ways the best solvent for polystyrene. A piece of petal may be passed directly from paraldehyde to Canada balsam. Venetian turpentine in paraldehyde seems to be as stable in the air of an ordinary room as a solution of Canada balsam in xylene. The precautions previously taken because of the sensibility of Venetian turpentine in absolute alcohol to air moisture thus become unnecessary.—*C. E. Allen.*

SPENCE, D. S. **Notes on mounting VII.** *The Microscope*, **4**, 266–72. 1941.

Styrax as a mounting medium was introduced by van Heurck in 1883. This name and "storax" have been applied to various substances. The styrax of the British Pharmacopoeia comes from a Turkish tree, *Liquidambar orientalis*; it is a pathological product obtained after the cambium has been wounded by beating the bark. The refractive index has been variously reported at from 1.5595 to 1.63.

Balsam of tolu is obtained by making incisions in the bark of *Myroxylon toluifera*. Its refractive index is given as 1.618. The following method of preparation suggested by C. H. Kain is recommended: Dissolve 3 oz. of the balsam in 4 fluid oz. of benzene; mix this at 45° C. with 4 fluid oz. of CS_2; shake well and after standing remove the CS_2 solution; repeat the process; evaporate the benzene, and add to the residue ⅓ its volume of α-monobromonaphthalene. After this treatment the refractive index is said to be 1.73.

Media of low refractive index are used in examining fibers of cloth. A medium is cited from Preston's *Modern Textile Microscopy* which consists of equal weights of low viscosity nitrocellulose, glycol diacetate, and acetone. This when set has a refractive index of 1.45. Other plasticizers than glycol diacetate give

media of higher refractive indices: triacetin, 1.47; resorcinol diacetate, 1.52; benzyl alcohol, 1.53; tricresylphosphate, 1.55; benzophenone, 1.57.—*C. E. Allen.*

DYES AND THEIR BIOLOGICAL USES

COPLEY, A. L., and WHITNEY, D. V. **The standardization and assay of heparin by the toluidine blue and azure A reactions.** *J. Lab. & Clin. Med.*, **28**, 762–9. 1943.

Either azure A or toluidine blue can be used for standardizing heparin by the following method: Titrate a measured portion of the heparin sample by adding 0.5 cc. increments from a 25 cc. burette, of a 1:2000 dilution of the dye (author used 0.8475 g. of toluidine blue MU-3, or 0.5747 g. of Azure A NAz 7 to 1000 cc. of water). Continue until a distinct blue solution appears among the heparin-dye floccules. Multiply the titration in milliliters by 3.33 to obtain the colorimetric unitage. (This was determined by comparison with the colorimetric technic.) This method is more accurate (to $\pm$ 5%) than the colorimetric method. One colorimetric unit of heparin decolorized 150 γ of the dye.—*John T. Myers.*

ANIMAL MICROTECHNIC

FRENCH, A. J. **Notes on the preservation, mounting and staining of Entomostraca.** *The Microscope*, **5**, 20–2. 1942.

The best preserving and mounting medium tried for Cladocera is an aqueous solution containing 30% glycerin and 3% formaldehyde. The mount should be in a ring of Murrayite well hardened on the slide, which ring receives a fresh coat of Murrayite before the cover slip is applied. The edge of the cover is washed and dried, and one or two rings of dammar in xylene are applied before the final ringing with Murrayite and marine glue. Collections may be killed in the field by adding 1/5 volume of formalin (40% formaldehyde) to the tube containing them. The specimens should be transferred as soon as convenient to the above-mentioned preservative.

Good results can be obtained by mounting Cladocera in a resinous material such as Canada balsam, euparol, or sira, by the following technic: Fix by adding to the water containing the living specimen an equal volume of a hot saturated solution of $HgCl_2$ with 2% acetic acid (or 1% acetic and 1% nitric acid). When the object becomes partially opaque, wash it once or twice in 20% alcohol tinged yellow with iodine. Pass it thru a series of alcohols from 30% to absolute (or cellosolve). Clear in creosote or clove oil by placing the specimen carefully in a small tube with an equal volume of absolute alcohol or cellosolve above, and allowing it to sink gradually. If clearing renders the object too transparent, add a drop or two of a filtered alcoholic solution of light green, eosin, or chlorazol black to the clearing agent. Transfer the cleared specimen with a pipette to the center of a slide. Remove excess of clearing agent with a fine pipette, and drop the mountant on the specimen. Place three small fragments of cover glass around it, and apply the cover.

Forms preserved in the glycerin-formaldehyde mixture also may be mounted in a resinous medium. Good mounts have been made of such material after 4 or 5 years in the preservative.—*C. E. Allen.*

GRUNEBERG, H. **The anaemia of flexed-tailed mice (Mus musculus L.). II. Siderocytes.** *J. Genetics*, **44**, 246–71. 1942.

Siderocytes in anemia have remained undiscovered because histochemical tests for iron have seldom been used in hematology. The following method of testing for iron gave good results: air-dry a blood film; fix it in absolute methyl alcohol; flood 1–3 min. with a freshly prepared solution of 1% HCl (the reaction does not occur in the absence of HCl); counterstain with Biebrich scarlet (apparently used in the same way as eosin in a blood stain). The siderophilic granules are blue, the cytoplasm various shades of pink. The granules fade when mounted with Canada balsam under a cover slip but can be restained.—*Virgene Kavanagh.*

MARKELL, E. K. **Gametogenesis and egg-shell formation in Probolitrema californiense Stunkard, 1935 (Trematoda: Gorgoeridae).** *Trans. Amer. Micr. Soc.*, **62**, 27–56. 1943.

The following suggestions are made for improving the fixation of flukes: Excess pressure should be avoided when cytological studies are to be made, because the cells can be greatly distorted. Gilson's fluid is much superior to Bouin's in the study of vitelline cells and shell formation. Silver impregnation (Bodian's method) followed by gold toning is very successful. Sections can be stained most satisfactorily with Heidenhain's hematoxylin when they have been mordanted 2 hr. with 5% aqueous ferric alum in 50% alcohol, washed quickly in alcohol, stained 9 hr. in 2% hematoxylin in 50% alcohol, and destained in a saturated solution of picric acid in 70% alcohol. Another procedure is to stain with azo-carmine and to counterstain with Roewer's methylene blue and ammonium picrate mixture; but directions are not given for this method.—*Virgene Kavanagh.*

PULLINGER, B. D. **A technique for the microscopic examination of the cornea.** *J. Path. & Bact.*, **55**, 97–9. 1943.

The advantages of the method to be described are based on: "(a) formalin fixation and frozen section ensure the least possible shrinkage; (b) sections cut with the knife edge starting at a tangent to the surface display cells and fibrillar laminae in their widest measurements; (c) silver impregnation reveals the processes of the corneal corpuscles, which are seldom well stained by the usual dyes; (d) the method is extremely simple, easy and reliable".

The procedure used is as follows: (1) Remove the whole eye. Inject the fixative (10% neutral formalin in distilled water) into the vitreous body and at the same time withdraw fluid from the chamber with another hypodermic syringe. Leave the whole eye in fixative 24 hr. This keeps the cornea in shape. (2) After 24 hr. cut the cornea off around the corneo-scleral margin. Detach the iris, ciliary body and lens. Keep the lens and fix separately. Fix the cornea an additional 3 days (4 days in all). (3) Nick the cornea on the edge opposite the point where sections are to be made, to avoid difficulty in locating this point when the cornea has been blackened by silver impregnation. (4) Transfer cornea to distilled water; avoid use of metal instruments. Wash with several changes. Leave overnight in distilled water to which 3 drops of NH_4OH (sp. gr. 0.88) have been added (volume of solution not stated). (5) Wash in 2–3 changes distilled water. (6) Pour off last change of distilled water and filter on to the cornea about 20 ml. of del Río-Hortega's silver carbonate solution. (7) Place dish containing the cornea in silver solution in a 37°C. incubator for 4 hr. (8) Remove cornea from incubator and allow to cool. Wash the blackened cornea in several changes of distilled water. (9) Pour neutral formalin in distilled water (1:10) onto the cornea to reduce the silver. A slight white cloud appears around the surface of the cornea. Leave specimen in formalin 15 min. The cornea is now ready to cut, but if the formalin is changed the specimen may be set aside, even for several weeks, until convenient to cut. (10) Cut sections with knife edge at a tangent to the surface. Support the curvature of the cornea during freezing. A lens cut in half and pared to fit forms a convenient support. (11) Freeze and cut sections; 15 μ is a satisfactory thickness. Freeze rather hard. Re-freeze when necessary, particularly just before reaching Descemet's membrane.

Sections are treated as follows: (1) Drop cut sections into distilled water by means of a glass rod or camel's hair brush. (2) Cover those to be examined at once with glycerin jelly, or pass thru graded alcohols to absolute alcohol. (3) Clear and soften in pure creosote, in which the sections may be left several hours or overnight. (4) Transfer section to glass slide on which is placed a few drops of creosote. Suck off excess creosote; remove remainder by tilting slide vertically. (5) Mount in a drop of balsam.

By this method, corneal collagen is a pale yellow, lighter in color than vascular cornea. While large nerve trunks are clearly shown, finer ones are revealed with greater regularity by preliminary treatment with 1.5% $AgNO_3$ for 24 hr. Fat in the cornea is also well demonstrated by appropriate stains after tangential frozen section.—*S. H. Hutner.*

RANDOLPH, THERON G. **Enumeration and differentiation of leukocytes in the counting chamber with propylene glycol aqueous stains.** *Proc. Soc. Exp. Biol. & Med.*, **52**, 20–2. 1943.

The most satisfactory results in enumeration and differentiation of leukocytes in the counting chamber are obtained when the blood to be examined is diluted with a solution of methylene blue dissolved in propylene glycol and mixed with aqueous eosin. The stock solutions used are (1) methylene blue, 0.1 g., in 100 ml. propylene glycol and (2) eosin B, 0.1 g., in 100 ml. distilled water. (Eosin Y is satisfactory but it stains eosinophile granules more lightly.) Equal parts of these two solutions are mixed and used as diluting fluid in the white blood cell pipette; after 24 hr. this combination is less effective as a nuclear stain. The mixture of blood and stain should remain in the pipette for 15 min. before filling the counting chamber.—*M. S. Marshall.*

RUTH, E. B. **A method for making sections of bones and teeth.** *J. Lab. & Clin. Med.*, **28**, 751–5. 1943.

The following procedure is recommended: Place the specimen of fresh, fixed, or dried bone in 5% HCl until decalcified (test each change of HCl for calcium by means of 5% NH_4OH and 5% ammonium oxalate). Wash in tap water until free from acid. Clear in hydroxide-glycerin solution (water 77 ml., glycerin 20 ml. and KOH 3 g.) and then in rising grades of glycerin (50%, 75%, and 100%). Wash in several changes of 70% alcohol, then with n-butyl alcohol. Embed in paraffin and cut sections, 40–200 μ thick, with a razor or microtome knife free-hand, or with a celloidin microtome. Place in butyl alcohol in a paraffin oven, and leave until the paraffin is dissolved, using 2 or 3 changes. Pass thru mixtures of butyl alcohol and glycerin containing increasing percentages of the latter (25%, 50%, 100%). Mount in glycerin jelly (USP gelatin 15 g., distilled water 50 ml., glycerin 55 ml. and phenol crystals 2 g.). Lamellae and lacunae are demonstrated in a striking manner and the unstained thick slides give a three dimensional picture.—*John T. Myers.*

SAYE, E. B. **Substitute for Wright's blood stain.** *Amer. J. Clin. Path., Tech. Suppl.*, **7**, 12–3. 1943.

To obviate the difficulty of obtaining methyl alcohol, the following technic is suggested: make blood film as usual and dry quickly in air; stain 1 min. in 0.25% alcoholic eosin (10 ml. saturated absolute alcoholic Coleman and Bell eosin Y + 90 ml. 95% alcohol); slowly lift slide vertically from the eosin solution with dry forceps and stain 1 min. in 0.05% aqueous thionin (20 ml. saturated alcoholic National Aniline thionin + 80 ml. tap water); remove slide as above and wash in tap water; dry in air or over a flame.—*L. Farber.*

SCHLEICHER, E. M. **Mallory-Heidenhain's connective tissue stain, employing domestic azocarmine B, aniline blue and orange G in balanced concentration.** *Techn. Bull. Registry of Med. Technologists*, **4**, 35–39. 1943.

The author has experienced difficulty in getting domestic azocarmine capable of giving exactly the results in the Mallory-Heidenhain technic which were secured with the foreign brand previously available. In a modification of the technic, however, he has obtained good results with either azocarmine B or azocarmine G of domestic manufacture (National Aniline), but prefers azocarmine B. The recommended procedure is:

Fix tissue in Helly's fluid for 5 hr. or less. Treat sections in Lugol's solution 2 min., in 1% $Na_2S_2O_3$ 3 min., in running water 10 min., then rinse in distilled water. Stain in 0.1% azocarmine B in 5% acetic acid; either stain wholly at room temperature, or begin at room temperature and transfer to 56° incubator, checking intensity of staining after 10 min. Rinse in distilled water and treat 15–30 min. in 5% phosphotungstic acid, until the connective tissue is faint red or colorless while the nuclei remain red, renewing the solution if it becomes bluish. Rinse in distilled water. Stain 15–30 min. in a staining fluid prepared as follows: have ready a stock solution of 0.2% anilin blue W.S. (certified batch No. NK-2 used), and 0.4% orange G (certified batch No. CO-4 used) in 1% acetic acid, and dilute one volume of this with two volumes of distilled water before using; check staining affinity frequently. Rinse in distilled water, then in 95%

alcohol until no more stain comes off. Dehydrate in absolute alcohol, one bath of 1 min. and a second of 5–10 min., checking frequently to be sure the anilin blue is not being extracted too greatly. Pass thru two changes of xylene and mount. Results (with kidney tissue): nuclei of glomerular epithelium, carmine; glomerular basement membrane deep blue; erythrocytes deep red; connective tissue blue.—*H. J. Conn.*

MICROÖRGANISMS

BAISDEN, L., and YEGIAN, D. **The destruction of acid-fastness of the tubercle bacillus by an autolytic process.** *J. Bact.*, **45**, 163–6. 1943.

Tubercle bacilli washed and suspended 1½ min. in 95% ethyl alcohol, and extracted with ether for 2 days at room temperature, remained acid-fast. If they were then incubated in water at 37°C. for 2 days at about pH 6, most of the acid-fastness was lost. This seemed to be due to autolysis, because heating to 90°C. for 10 min. or incubation at 50° C. prevented the loss of acid-fastness and incubation at 10°C. reduced it greatly.—*Virgene Kavanagh.*

GOLDSMITH, J. B. **A simple method for differential staining of fecal smears containing protozoan parasites.** *Trans. Amer. Micr. Soc.*, **62**, 94–5. 1943.

Fecal smears seldom show differentiation in staining between fecal material and protozoan parasites, but the following method leaves fecal material cream-color or tan, and protozoa purple to blue-black: Prepare a smear by mixing a bit of fecal material with a drop of water on a slide. Kill the protozoa by flaming. Dry until only a trace of moisture remains in the center of the drop. Fix 12–24 hr. in chrome-acetic-dichromate (15 vol. 1% aqueous CrO_3, 4 vol. 2% aqueous $K_2Cr_2O_7$, 1 vol. glacial acetic acid) diluted with an equal vol. of water. Stain with Heidenhain's iron hematoxylin by the short method. (Fixative and stain both in Guyer, 1930) —*Virgene Kavanagh.*

GRACIAN, M. (Inst. Hig. Milit., Madrid.) **Ein einfaches Verfahren zur Färbung der Rickettsien.** *Zts. Hyg. Infektionskr.*, **124**, 81–2. 1942.

The dried smear is treated for 3 min. with xylene, the xylene is poured off, the slide is (without drying) flooded twice with 96% alcohol and washed with tap water. It is then flooded for 3 min. with a saturated solution of $K_2Cr_2O_7$, thoroly washed with tap water, and stained for 10 to 20 min. with a 10% Giemsa solution (2 drops to 1 ml. of distilled water). It is finally washed with tap water and dried.—*Theodor von Brand.* (From Biological Abstracts.)

KATZNELSON, H. **A staining technique for evaluating the toxicity of an antibiotic substance of microbiological origin.** *Canad. J. Res.*, Sec C, **20**, 602–8. 1942.

The toxicity of an antibiotic agent produced by a microörganism was measured by determining the per cent of cells of *Schizosaccharomyces pombe* which were killed by it. The dead cells were identified by the fact that they stain with neutral red, whereas living cells are unstained. The procedure used is: grow the yeast on nutrient agar containing 1% peptone and 2% dextrose for 24 hr. at 28°C.; suspend cells in potato dextrose broth at pH 6.3; by means of a counting chamber standardize suspension so that 1 ml. contains 160,000,000 cells; to 1 ml. of toxic test material in a glass tube 2½ × ⅜ in. add 0.5 ml. of cell suspension and 0.5 ml. of 1:5000 aqueous neutral red; plug the tube; shake thoroly; place in water bath at 36°C. for 3 hr.; agitate intermittently; remove from water bath; shake; transfer some of contents to a counting chamber; count stained and unstained cells in 20 of smallest squares; express results in per cent of stained cells.—*H. P. Riley.*

KELSO, R. E., and GALBRAITH, T. W. **A rapid simple technic for detection of acid fast bacilli in urine.** *Amer. J. Clin. Path., Tech. Suppl.*, **7**, 8–11. 1943.

The procedure recommended is as follows: Collect about 500 ml. of urine, including morning specimen; shake well and place 350–400 ml. in a 500 ml.

volumetric flask; add 3–4 ml. toluene and shake vigorously for 5 min.; add enough freshly distilled water to bring the fluid to within 1½ in. of the top of the flask; allow to stand at least 6 hr.; with a long-handled loop go below the bulk of the scum near the junction of the foam and liquid layers; move the loop around at this level so as to pick up as much as possible; bring loop out without turning; spread 3–5 loopsful over a new clean slide; air dry, fix in flame and stain by Ziehl-Neelsen method; examine with oil immersion lens.—*L. Farber.*

MARKEY, R. L., CULBERTSON, C. S., and GIORDANO, A. S. **Rapid method for the staining of intestinal parasites.** *Amer. J. Clin. Path., Tech. Suppl.*, **7**, 2–3. 1943.

The following method is recommended for staining the cysts and trophozoites of amoebas (*Chilomastix*, and *Giardia*) and trichomonas: Make smears of fresh stool and, while wet, place 2 min. or more in Schaudinn's fluid (saturated aqueous $HgCl_2$, 2 parts, + 95% alcohol, 1 part + glacial acetic acid, 10% by volume); immerse 15–30 sec. in 95% alcohol (for permanent mounts remove $HgCl_2$ with iodine-alcohol); rinse in water; treat 2–3 min. with iron-alum (5.0 g. Fe $NH_4(SO_4)_2 \cdot 12\ H_2O$ in 100 ml. distilled water) at 56°C.; rinse in water; stain 1–2 min. at 56°C. with hematoxylin solution (10% alcoholic hematoxylin, 0.4 ml. + glacial acetic acid, 0.8 ml. + distilled water, 40 ml.); rinse in water and allow to stand in air until blue-black (for permanent mounts wash 15–30 min. in running water); dehydrate 30 sec. to 1 min. in 95% alcohol, followed by 1 min. in absolute alcohol or acetone; clear in xylene; mount in clarite, Canada balsam or gum dammar.—*L. Farber.*

POTHMANN, FR. J. **Vergleichende Untersuchungen mit der Leuchtbildmethode und dem Fluoreszenzmikroskop zum Nachweis der Tuberkelbazillen bei gleichzeitiger Kontrolle im Hellfeld.** *Arch. Hyg. Bakt.*, **125**, 148–61. 1940.

A comparison was made of the relative merits of dark-field and fluorescent microscopy for the detection of tubercle bacilli. In 245 streak-preparations of sputum, feces, and gastric juice, 2250 visual fields were examined; the fluorescent microscope made visible 20,415 bacilli, while the dark-field with Ziehl-Neelson staining yielded 15,503, a ratio of 1.3:1 in favor of the fluorescent microscope. In the dark-field, however, with the Osal method of staining, a better picture was obtained and the yield was but 1/10 less than with the fluorescent microscope. The ordinary bright-field showed only about half the number of bacilli appearing in the dark-field, and two of the preparations which appeared negative in the bright-field proved to be positive when examined by the other two methods.

For fluorescent microscopy, an ordinary projection condenser was used, since the quartz collector held no advantage between the wave lengths of 300 mμ to 400 mμ. An ordinary microscope with 1.4 Abbé lighting apparatus was used with compensating oculars 5 and 8, and an apochromatic objective of 60× and N. A 0.95 without corrected cover glass. A Zeiss automatic arc lamp of 10 amperes served as a light source. The streak-preparations were made upon ultraviolet-transparent glass slips 1.5 mm. thick and were stained for 15 min. in a 1:1000 auramine solution in 5% phenol, and, after thoro washing with tap water for 3 min., were differentiated in 95% alcohol to which 4% NaCl and 4% HCl were added. This was followed by repeated, thoro washing and drying. Counterstaining appeared to be disadvantageous.

For the dark-field method, the Leitz dark-field condenser N.A. 1.2 and the Periplan oculars of Leitz were used. Then, by means of the Zeiss objective 90× with iris diaphragm, giving numerical apertures of 1.25 to 0.8, the preparation could be viewed either in dark-field (with the diaphragm closed, i. e. at 0.8) or in bright-field with the diaphragm 1.25. The 250 c.p. Osram-Nitra lamp served as a light source with a flask of water placed in the light path to concentrate, cool, and diffuse the light which must cover the entire surface of the plane mirror. The condenser was covered with water or oil and the preparation, spread sufficiently thin and stained either by the method of Ziehl-Neelson or of Osal, was laid on carefully to avoid air bubbles, and focused so as to be uniformly lighted.

In the alternative staining method of Osal, the streak-preparations were briefly boiled two or three times in carbol-fuchsin, bleached in 5% H_2SO_4 and

washed in water, then treated with 10% Na_2SO_3 solution, to 100 ml. of which was added 20 cc. of absolute or 96% alcohol.

The dark-field method proved simpler, less expensive and less restricted in use, than the fluorescent microscope.—*Merritt N. Pope.*

SMITH, W. W. **Action of alkaline acriflavine solution on Bacterium salmonicida and trout eggs.** *Proc. Soc. Exp. Biol. & Med.*, **51**, 324–6. 1942.

Acriflavine, pH 7.7, in a concentration of 500 p.p.m. killed *Bacterium salmonicida* on the shell of trout eggs in 20 min. This solution did not injure the eggs after exposure of half an hour. The disinfection of eggs in conjunction with other quarantine procedures was designed to reduce furunculosis in trout, a costly, wide-spread, epizootic bacteremia.—*M. S. Marshall.*

VAN DYKE, ANNE E. **A comparative evaluation of Ziehl-Neelsen and fluorescent methods in demonstrating acid fast bacilli.** *Amer. J. Clin. Path., Tech. Suppl.*, **7**, 6–8. 1943.

Examinations for tubercle bacilli were made in 360 smears by the Ziehl-Neelsen and fluorescent methods. For best results smears should be thoroly decolorized and examined in a dark room with a shielded lamp. The fluorescent method proved the more efficient as to time consumed and results obtained in examining smears for tubercle bacilli.—*L. Farber.*

STOWELL, R. E. **Photometric histochemical determination of thymonucleic acid in experimental epidermal carcinogenesis.** *J. Nat. Cancer Inst.*, **3**, 111–21. 1942.

A description is given of a microphotometric apparatus. The procedure recommended employs a modification of the Feulgen technic in which the relative amounts of thymonucleic acid at various stages of methylcholanthrene carcinogenesis in the skin of mice are estimated. The instrument is also designed to measure the amounts of stain and pigment in tissue sections. The technic is so devised that a relative specific stain for one tissue component enables one to determine comparatively the amount of the stain in the tissue.—*P. G. Roofe.*